# Cooperation and Exploitation in Communally Nursing House Mice (*Mus musculus domesticus*)

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*The road to wisdom? – Well, it's plain and simple to express:*
*Err and err and err again but less and less and less.*

*— Piet Hein*

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# SUMMARY

Cooperative behaviours are ubiquitous in nature and puzzle scientists ever since Darwin formulated his theory for evolution through natural and sexual selection. Why should an individual help another one, if selection favours those maximising their own fitness? Indirect fitness benefits as first described by Hamilton, and alternatively, direct fitness benefits for cooperating individuals (for example through reciprocity) helped to explain the evolution of cooperation over the last 60 years. Understanding the evolution and maintenance of cooperative behaviours in nature nevertheless requires identifying the potential for conflict and the mechanisms in place to prevent exploitation.

This thesis focuses on communal nursing in house mice (*Mus musculus domesticus*). Female mice show two alternative reproductive tactics; rearing their young either solitarily, or pooling their litters in one nest and caring for all pups indiscriminately. Communal breeding can be observed in many different species, which is remarkable given the high costs of parental care. The potential for exploitation seems high, whenever females differ in the amount of care they provide to the joint litter, or the number of young they have in the nest. I combined experiments in the laboratory with data from a free living population to asses whether there is potential for conflict and to quantify the fitness consequences of the two alternative reproductive tactics in house mice.

Communally nursing females invested according to the total number of pups in the nest, but not their own litter size, making them highly vulnerable to exploitation (Chapter 1). Accordingly, females with relatively fewer offspring in the nest overinvested. In the laboratory, females tried to avoid situations of high conflict by reducing their propensity to cooperate when females differed in litter size (Chapter 2). Furthermore, I found no evidence that females enforced their partner's continued investment (Chapter 3). Overall, it seems females had only limited options to reduce the risk for exploitation after the formation of the communal nest, emphasising the importance of deciding beforehand whether, or with whom, to communally nurse.

Data from a wild population revealed that communally nursing females suffered an increased pup mortality, resulting in a lower reproductive success for females rearing a larger proportion of their litters communally (Chapter 4). Older, and probably heavier females, were more likely to rear their litters solitarily, indicating that it represented a condition dependent alternative reproductive tactic. Younger females may have been unable to rear litters solitarily, therefore opting for communal nursing as a "best-of-a-bad-job", even at the cost of losing some of their offspring.

The research presented in this thesis demonstrates the strong potential for conflict among communally breeding females and reveals that an apparent cooperative behaviour does not necessarily always result in benefits for all individuals

involved. Plastic and condition dependent alternative reproductive tactics might nevertheless maintain such behaviours in the population, highlighting the importance for future research about the evolution of plasticity and its effect on cooperation.

# ZUSAMMENFASSUNG

Seit Darwin seine Theorie der Evolution durch natürliche und sexuelle Selektion entwickelt hat, stellt sich die Frage nach dem Ursprung von kooperativem Verhalten. Weshalb sollte ein Individuum einem anderen helfen, wenn Selektion jene bevorzugt, welche ihre eigene Fitness maximieren? Die Möglichkeit indirekter Fitnessgewinne nach Hamilton und alternativ, direkte Fitnessvorteile (beispielsweise durch Reziprozität) halfen unser Verständnis über die Evolution von kooperativem Verhalten in den letzten 60 Jahren zu vertiefen.

Um die Evolution eines kooperativen Verhaltens zu verstehen, ist es allerdings unabdingbar das Konfliktpotential zu erkennen und die Mechanismen zu identifizieren, welche die Kooperation stabilisieren.

Diese Dissertation beschäftigte sich mit gemeinschaftlicher Jungenaufzucht in der westeuropäischen Hausmaus (*Mus musculus domesticus*). Weibchen dieser Art zeigen zwei alternative Fortpflanzungstaktiken; sie können ihre Jungtieren entweder alleine grossziehen, oder aber zusammen mit einem oder mehreren anderen Weibchen. Unter diesen Umständen säugen die Weibchen alle Jungtiere im Nest. Gemeinschaftliche Jungenaufzucht lässt sich in einer Vielzahl von Arten beobachten, was bemerkenswert ist, bedenkt man die hohen Kosten, die mit elterlicher Pflege einhergehen. Das Konflikpotential scheint gross, sobald die Weibchen nicht gleich viel in das gemeinschaftliche Nest investieren, oder sich in der Anzahl an Jungtieren unterscheiden. In dieser Dissertation kombinierte ich Laborexperimente mit Daten einer freilebenden Mäusepopulation, um das Konfliktpotential abzuschätzen und die Fitnesskonsequenzen der zwei alternativen Fortpflanzungstaktiken zu quantifizieren.

Weibchen die ihre Jungen gemeinsam aufzogen, investierten gemäss der Gesamtanzahl Jungtiere im Nest und nicht ihrer eigenen Wurfgrösse, was sie sehr anfällig dafür machte, ausgebeutet zu werden (Kapitel 1). Demnach investierten Weibchen überproportional viel, die im Verhältnis weniger Jungtiere im Nest hatten als ihre Partnerin. Im Labor versuchten die Mäuse solchen Situationen mit grossem Konfliktpotential aus dem Weg zu gehen; sie zeigten eine verringerte Wahrscheinlichkeit ihre Jungen gemeinsam aufzuziehen, wenn die Weibchen sich in ihren Geburtswurfgrössen markant unterschieden (Kapitel 2). Des Weiteren fand ich keine Hinweise darauf, dass die Weibchen ihre Investition reduzierten, wenn sie weniger Junge im Nest hatten, oder dass sie durch aggressives Verhalten versuchten, ihre Partnerin ihrerseits an einer Reduktion der Investition zu hindern (Kapitel 3). Generell scheinen Weibchen nur sehr eingeschränkte Möglichkeiten zu haben, eine Ausbeutung zu verhindern, sobald das Gemeinschaftsnest erst einmal besteht. Sehr viel wichtiger scheint also die Entscheidung, ob und mit wem die Weibchen die gemeinschaftliche Jungenaufzucht eingehen.

Daten einer freilebenden Population zeigten, dass Weibchen, die einen grösseren Anteil ihrer Würfe gemeinschaftlich aufzogen, einen tieferen Fortpflanzungs-

erfolg hatten, darauf beruhend, dass Jungtiere im Gemeinschaftsnest eine geringere Überlebenswahrscheinlichkeit hatten (Kapitel 4). Ältere und daher vermutlich auch schwerere Weibchen zogen einen grösseren Anteil ihrer Würfe solitär auf, was darauf hindeutet, dass es sich dabei um eine situationsabhänge Fortpflanzungstaktik handelt. Im Gegensatz zu älteren Weibchen schienen Jüngere es schwerer zu haben ihre Jungen alleine aufzuziehen und wählten deshalb, in dem Versuch das Bestmögliche aus ihrer Situation herauszuholen, die gemeinschaftliche Jungenaufzucht als Alternative, auch wenn sie dadurch ihn Kauf nahmen einige ihrer Jungtiere zu verlieren.

Die Ergebnisse dieser Dissertation verdeutlichen, wie stark das Konfliktpotential unter gemeinschaftlich aufziehenden Weibchen ist und offenbaren, dass ein anscheinend kooperatives Verhalten nicht immer für alle Beteiligten von Vorteil sein muss. Plastische und situationsabhänge alternative Fortpflanzungstaktiken können uns dabei helfen zu erklären, weshalb solch ein Verhalten trotz Fitnesskosten in der Population erhalten bleibt, und verdeutlichen damit, wie wichtig die weitere Erforschung der Evolution von Plastizität und insbesondere deren Einfluss auf kooperatives Verhalten ist.

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# GENERAL INTRODUCTION

# GENERAL INTRODUCTION

*"If it could be proved that any part of the structure of any one species had been formed for the exclusive good of another species, it would annihilate my theory, for such could not have been produced through natural selection."*

*— Charles Darwin (1859)*

## THE EVOLUTION OF COOPERATION

Cooperative interactions can be observed in many different species and contexts all throughout the tree of life. From fruitingbody forming bacteria (Velicer and Vos, 2009) to the complex symbiotic partnerships between plants and fungi (Frank, 1885), cooperation is ubiquitous in nature and has always caught people's attention. Ever since Darwin formulated his theory for evolution through natural and sexual selection, the evolution of cooperation marked one of the big questions in biology. Cooperation is defined as an act performed by an individual that benefits one or several other individuals (Sachs et al., 2004). Why should an individual help another one, if selection favours those maximising their own fitness? Cooperation seems very vulnerable to be invaded by a cheating strategy, because defectors that do not show the cooperative behaviour can benefit from the cooperation of others without having to pay any of the costs associated with it (Nowak, 2006).

Gene, rather than individual based selection, as first described by Fisher (1930) and further applied to the evolution of cooperation by Hamilton (1964a,b) provided one possible solution to the problem. If individuals help those individuals with whom they share a large proportion of their genes, they can increase their fitness indirectly by promoting the spread of the genes through the offspring of their relatives (or generally through individuals that share the same genes).

The alternative scenario under which the evolution of cooperation is expected to be stable is when individuals gain direct benefits from the cooperation, as for example by only cooperating with individuals that return the favour (*Reciprocity*; Trivers (1971)). Though the last 60 years went a long way of explaining the evolution and maintenance of cooperation, the question is still heavily studied. The journal *SCIENCE* in 2005 at its 125th anniversary still considered "how did cooperative behaviour evolve" to be one of the 125 most important questions in science (Pennisi, 2005).

### *Cooperative offspring care*

One intriguing area of cooperation is cooperative offspring care, which is defined as individuals caring for young that are not their own. Cooperative care of young can be observed in most clades that show parental care; it has been described

for insects, social spiders, birds, mammals and fish (Taborsky, 1984; Eggert and Müller, 1992; Solomon and French, 1997; Koenig and Dickinson, 2004; Salomon and Lubin, 2007). Between species rearing their young cooperatively, the reproductive skew can vary extensively. On one hand of the spectrum we have cooperatively breeding species with only a dominant female or dominant pair breeding and no subordinate individuals reproducing (mammals: (Solomon and French, 1997), birds: (Brown, 1987), fish: (Taborsky, 1984), insects: (Michener, 1969). The higher the number of successfully reproducing subordinates, the more egalitarian the system becomes until all females of a group reproduce, as it is the case in communally breeding species. Examples for communally breeding species include subsocial spiders (Schneider, 2002), banded mungooses (Hodge et al., 2009) and Acorn woodpeckers (Mumme et al., 1988).

Females rearing their young communally pool their young in one nest and share their maternal load. Several benefits were associated with communally rearing offspring. Having two or more females defending the nest could decrease predation or infanticide by conspecifics. Furthermore, females could share the costs of building a nest and a larger number of offspring in the nest might facilitate thermoregulation (Hayes, 2000). However, females sharing a nest might also compete over resources, larger nests might be more conspicuous to predators, and the larger number of individuals might increase the risk for the transmission of parasites and diseases (for a review see (Hayes, 2000).

At first sight, and in contrast to cooperative breeders, where reproduction is monopolised by dominant individuals, communal breeding or joint nesting appears to be less ridden with conflict. All females involved reproduce and therefore gain direct fitness benefits. However, a closer look reveals in fact several ways in which communally breeding females could gain benefits at the cost of their group members. Cooperation between two ore more individuals provides the potential for one exploiting the others. Specifically, conflict among communally nursing females is expected to manifest itself in two ways - i) females might differ in the amount of energy (i.e. milk) they invest into the nest and ii) they might have varying numbers of pups in the joint litter. In the following I will discuss both of those aspects and how they are connected.

**Female investment** The first potential area of conflict is the amount of female investment. By pooling their litters in one nest, females likely will not only show maternal care towards their own, but also alien offspring. Since parental care is associated with high costs (Clutton-Brock, 1991), misdirecting it towards non-offspring, especially when dealing with altricial offspring, should be avoided. There are some communally breeding species in which females preferentially care for their own young, thereby reducing or bypassing the high costs (Pusey and Packer, 1994; Jesseau et al., 2009). The potential for conflict between females in those instances seems small, because all females invest according to their own contribution to the communal litter/joint nest. However, in a number of communally breeding species females appear to indiscriminately care for all offspring in the nest, providing a scope for exploitation (Watkins and Shump, 1981; Holmes and Sherman,

1982; Koford et al., 1990; Samuk and Avilés, 2013). Depending on how much the females invest and how many offspring they have in the joint nest, they may over- or underinvest. Females should therefore have a selfish incentive to decrease their investment at the cost of the other group members, analog to a classic "public goods" problem, with the costs of one individual investing less being shared equally by all individuals involved (Rankin et al., 2007).

**Pay-off - number of young in the communal nest** While communally breeding species are characterised by all involved females reproducing, there might still be differences in the number of offspring the females wean, which presents the second area of conflict. We cannot look at this independently of female investment, because the risk for exploitation is especially pronounced in species in which females indiscriminately care for all young in the nest. As discussed above, if all females invest equally, females with more offspring in the nest will underinvest at the cost of their partners. The costs of investment will be shared equally among all females, but the benefits will differ between the females depending on their contribution to the communal litter/brood (i.e. the number of young they have in the nest).

Communal breeding therefore, although potentially conveying a number of benefits to females, also comes with the potential for exploitation. Some females might reap larger benefits than they should based on their investment. The extreme of this would be conspecific brood parasitism, with females adding offspring to another female's nest without providing any maternal care themselves. In order to maximise reproductive success, different female tactics should have been selected for in different species/contexts, depending on the costs of rearing offspring solitarily vs. communally and depending on whether females can avoid being exploited during communal breeding. To understand what led to the evolution of a cooperative behaviour as communal breeding we therefore need to understand not only whether there is scope for exploitation, but also the benefits and costs associated with the different behaviours.

*Communal nursing in house mice*

In house mice (*Mus musculus domesticus*), females show two different breeding strategies. They were described to rear their young solitarily or communally together with one or several other females both under laboratory conditions and in wild populations (Sayler and Salmon, 1969; Wilkinson and Baker, 1988; König, 2006). As a small and fast reproducing mammal, mice are a particularly well suited study species to look at communal breeding. Lactation is very costly in mammals, raising the overall energy demand of lactating female house mice by 203% in comparison to control females (Speakman and McQueenie, 1996). Such high costs make the occurrence of allonursing - i.e. females nursing young that are not their own - even more striking and require an adaptive explanation.

Female house mice engaging in communal nursing pool their litters in one nest and are apparently not able to discriminate between own and alien offspring.

König (1989a) showed in a laboratory experiment under restricted feeding, that when females were forced to sacrifice part of their litter to be able to raise at least some young, they were equally likely to kill own versus alien offspring; a strong indicator that they are not able to discriminate. Communal nursing in house mice was associated with a number of benefits both in the laboratory and the wild. Firstly, females allowed to rear their young communally together with a sister had a higher lifetime reproductive success over an experimental lifespan of 6 months in the laboratory. Secondly, there is evidence from a semi-natural and natural population that pups reared communally are better protected against infanticide by conspecifics (Manning et al., 1995; Auclair et al., 2014b). Thirdly, it was suggested that communal nursing might improve pups' immune system, because they receive milk and therefore likely antibodies from more than one female (Roulin and Heeb, 1999). Lastly, communally rearing their young with another female further was shown to allow females to leave the nest more often, giving them more time for other actives as feeding or nest defence (Auclair et al., 2014a).

However, there is also evidence that communal nursing in house mice might not always be beneficial. Infanticide has been found to occur among communally nursing females (König, 1994a; Palanza et al., 2005; Schmidt et al., 2015) and could influence female success. Moreover, while receiving milk from several females might help to improve a pup's immune response, it might also increase the risk of contracting a disease. Overall, the higher number of both young and adults in the nest could increase parasite and disease occurrence and transmission.

As discussed above more generally, females might also run the risk of being exploited by their communal nursing partner as soon as females either differ in the amount of energy (i.e. milk) they invest into, or the number of offspring they contribute to the nest. To understand whether females are at risk of exploitation we first need to assess how they invest into the communal litter. In Chapter 1 of this thesis, I test whether females produce milk (as a proxy for energy invested) according to their own litter size, or the total number of pups in the nest - which would make them highly vulnerable to exploitation. Chapter 3 equally focuses on female investment, by testing whether females prevent each other from reducing their investment, which would be another way how females could benefit at the cost of the other female in the nest.

While Chapters 1 and 3 cover the potential for conflict evoked by variation in females' investment, Chapter 2 instead focuses on the second area of conflict; the number of pups a female has in the communal liitter. Assuming that females invest equally, the number of offspring has a huge effect on female success. The more pups a female has in comparison to her partners, the higher her benefit because she underinvests relative to the other female. Females therefore should have an incentive to decrease their partner's litter size (either through infanticide or by choosing a female that gave birth to an already smaller litter), and we would expect that in situations with large differences in litter size between females, the conflict should be highest and cooperation least likely to be successful. Chapter 2 tests under laboratory condition whether litter size differences between females influence a female's likelihood to cooperate, i.e. to communally rear her young.

Communal nursing in house mice is not obligatory. Females can also rear their young solitarily and were found to do so in the wild, even if they had the opportunity for communal nursing (females of their social group had dependent offspring at the time they were giving birth) (Weidt et al., 2014). It is therefore necessary to take solitary nursing into account when analysing communal nursing. While Chapter 1 already compares the investment (milk production) of communally and solitarily nursing females, Chapter 4 focuses more specifically on the consequences of communal vs. solitary breeding by analysing long term data from a wild population.

This thesis therefore aims to analyse the potential for exploitation among communally nursing female house mice by both conducting experiments in the laboratory (Chapters 1, 2 and 3) and quantifying the occurrence and fitness consequences of communal nursing in a wild population in Chapter 4. These new insights might help to identify the conditions favouring the evolution of communal breeding and deepen our understanding of the mechanism at work to prevent females from exploiting each other, which would jeopardise the evolution of cooperation.

# CHAPTER I

# 1

# THE RISK OF EXPLOITATION DURING COMMUNAL NURSING IN HOUSE MICE, *MUS MUSCULUS DOMESTICUS*

Manuela Ferrari, Anna K. Lindholm, Barbara König

## ABSTRACT

Parental care is associated with costs. Communal offspring care in species with altricial young may reduce the costs for a parent, but it comes with a risk of exploitation, jeopardizing the evolution of stable cooperation. Female house mice can either rear their young alone or communally with one or several other females. In the latter case, females pool litters and do not discriminate in their maternal behaviour between their own and alien offspring. Differences in litter size between females, or differences in the amount of investment they provide, might therefore result in one female exploiting another. To analyse the potential for conflict during cooperation, we compared under laboratory conditions the maternal investment (milk quantity and quality at peak lactation, when a female's own offspring were 15 days old) of wild-bred females nursing communally with one partner with that of females nursing solitarily. To increase the probability of asymmetry in litter sizes between communally nursing females, we used a genetic tool to reduce in utero litter size for one of the two partners. Communally nursing females invested according to the total number of pups in the joint nest and not according to their own litter size, making them vulnerable to exploitation. Females that gave birth to the smaller litter consequently overinvested; they had a higher investment per weaned offspring than females that gave birth to larger litters in communal nests or solitarily nursing females. Communal nursing in house mice thus represents a public good situation. Both partners invest according to the combined litter size, but they differ in the benefit they gain, which is the number of weaned offspring.

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## INTRODUCTION

Parental care improves offspring survival, typically at a cost for the investing parent in terms of decreased future reproduction or survival (Clutton-Brock, 1991). It is often associated with high energetic and opportunity costs, especially in species that produce altricial offspring, which initially fully depend on care or protection provided by adults (Clutton-Brock, 1991; Bennett, 1981). Communal offspring care may present an opportunity to reduce the costs of parental care and has been de-

scribed for about 15% of mammals (Gittleman, 1985; Bronson, 1989; König, 1997) and 2.5% of birds (Brown, 1987).

Communal offspring care can be expressed in two ways. First, nonreproducing individuals help to raise the offspring of other individuals. Second, several reproducing individuals share parental load by pooling their clutches or litters in one nest. The main difference between these types of communal offspring care is the degree of reproductive skew between females in a group. The skew can range from despotic, with one dominant breeder as in bird species with helpers-at-the-nest (Koenig and Dickinson, 2004) and in cooperatively breeding mammals (Clutton-Brock, 2002), to egalitarian reproduction among the females in a group as in communally (plurally) breeding species (Koford et al., 1990; Hayes, 2000). Although egalitarian groups may also experience reproductive skew, it will be less pronounced than in despotic groups. Lower potential for conflict is thus expected in communally breeding species, since all group members gain direct fitness benefits. If individual investment (cost), however, corresponds to the total number of offspring in the communal or joint nest and not a female's own offspring only, the potential for exploitation, and thus conflict, is raised also among communally breeding species.

Whenever we observe regular and indiscriminate provisioning of a female's own and alien offspring we have to analyse the underlying potential for conflict among the partners involved to understand the factors stabilizing cooperation during communal offspring care. Indiscriminate care or the lack of discrimination between a female's own and alien young in species with altricial offspring is rather common and has been described for a number of communally nursing mammals (bats: Watkins and Shump (1981); rodents: Holmes and Sherman (1982), communally feeding birds (Koford et al., 1990) and invertebrates (Samuk and Avilés, 2013).

Mammals provide interesting case studies for the potential for exploitation in communal offspring care. Females predominantly or exclusively provide parental care and lactation comes at a high cost (Bateman, 1957; Clutton-Brock et al., 1989). Lactation increases a mother's daily caloric intake by 66 up to 180% in comparison to the nonreproducing period (Gittleman and Thompson, 1988; König et al., 1988), and increased investment in the present offspring delays the birth of the next litter (Clutton-Brock et al., 1989). Regular and indiscriminate provisioning of milk to a female's own and alien offspring, as observed in communally nursing species, thus requires an adaptive explanation (Roulin, 2002; König, 2006) In house mice, allonursing of pups by communally breeding females has been observed both in the wild and under laboratory conditions (Sayler and Salmon, 1971; Wilkinson and Baker, 1988; Weidt et al., 2014; Schmidt et al., 2015). Communal nursing with a familiar partner improves a female's lifetime reproductive success analysed for wild house mice under laboratory conditions (König, 1994a). However, communal nursing in mice is facultative. Even during periods of high population density females nurse litters solitarily. When nursing communally they are selective, with clear evidence for social partner choice both in a free-living population and under standardized laboratory conditions (Weidt et al., 2007, 2014). In a laboratory exper-

iment, such social partner choice improved female lifetime reproductive success (Weidt et al., 2014).

Since litters in communal nests vary in age and in size, indiscriminate nursing has the potential for exploitation or free riding (when one partner benefits more than it invests, or even benefits without investing at all). If one female has a larger litter than her nursing partner(s), but all females invest equally in the combined nest, she will exploit the other(s). The benefit (number of offspring weaned) will vary for the different females contributing to the communal nest, while all partners share the costs (energy invested) equally. The risk of exploitation is even higher, as female infanticide occurs regularly among communally nursing females. The female that gives birth second may kill one or more of the other female's pups before she gives birth herself, therefore biasing the relative contribution to the communal litter in her favour (König, 1994a; Palanza et al., 2005).

Females could avoid being exploited by preferentially nursing their own young. Contrasting results have been found as to whether female house mice are able to discriminate between their own and alien pups in a communal nest, with overall only weak evidence indicating the ability to recognize their own offspring (Auclair et al., 2014a; Chantrey and Jenkins, 1982; Hager and Johnstone, 2005; König, 1989a; Manning et al., 1995; Yamazaki et al., 2000). Still, even an ability to discriminate between her own and alien young on the mother's side would not guarantee selective nursing. Females may be unable to fend off alien offspring in the confined environment of a communal nest (milk theft). To our knowledge, it has not yet been demonstrated or tested whether wild house mice are able to transfer more milk to their own than alien offspring, for example by allowing their own young access to teats with higher milk let-down. Furthermore, females in a laboratory study were not found to spend more time nursing their own versus alien young (König, 1989b). If females indeed selectively nurse their own young, we expect their milk production to correlate with their own litter size at the time of measuring milk production.

Alternatively, females could avoid exploitation by adjusting their milk production to the litter size to which they gave birth. However, female house mice adjust their investment to postpartum changes in litter size (Knight, 1982; König et al., 1988), very likely directly influenced through the number of suckling young. It has been observed in many mammals that milk yield increases with the number of sucklings (sheep, *Ovis aries*: Alexander and Davies (1959), 1959; goats, *Capra aegagrus hircus*: Hayden et al. (1979); rats, *Rattus norvegicus*: Morag et al. (1975), mice: (Knight, 1982; König et al., 1988)). If the suckling stimulus determines milk production, and if females are unable to prevent alien young from accessing their teats, we expect females to invest according to the joint litter size in the nest, irrespective of a female's ability to recognize her own offspring. Such indiscriminate nursing would make them highly vulnerable to exploitation, as soon as communally nursing females differ in litter size.

In a laboratory setting we analysed female investment during peak lactation (milk quantity and quality) in wild house mice to assess, first, whether communally nursing females invest according to their own litter size or the joint litter

size in the nest. To increase the probability of asymmetry in litter sizes between communally nursing females, we used a genetic tool to reduce in utero litter size for one of the two partners and thus avoided the disadvantages of manipulating litters shortly after birth (Ferrari et al., 2014). In a second step we compared the lactation performance of communally and solitarily nursing females to analyse whether females use different investment strategies under these different breeding conditions. Information about the potential of conflict among partners will be a prerequisite for understanding the factors stabilizing cooperation.

## MATERIAL AND METHODS

### *Animals and Husbandry*

Experimental animals were F1 to F3 descendants of wild house mice from a population near Zurich, Switzerland (for more information see König and Lindholm (2012)). Mice were kept in the laboratory at a temperature of 22–24 °C under a constant light:dark cycle of 14:10 h (light on at 0530 hours CET). Food (laboratory animal diet for mice and rats, no. 3430, Kliba) and water were provided ad libitum, as well as paper towels and cardboard that served as nest-building material. Experimental animals originated from monogamous breeding pairs and stayed in their parents' cages until the age of 28 days, when a tissue sample (small ear punch) was taken for genotyping and individual identification. Subadults were afterwards kept in same-sex sibling groups in Macrolon Type III cages (23.5x39 cm and 15 cm high) until the beginning of the experiment.

The population of origin contained a selfish genetic element, the *t* haplotype (Lindholm et al., 2013). This haplotype is characterized by drive in males (90% of offspring sired by a *t* heterozygous male inherit the *t*) and is associated with a recessive lethal, as has been described for other populations (Silver, 1993). Embryos that are *t* homozygous die in utero, so that a mating between two *t* heterozygous individuals results in 40% smaller birth litter sizes (Lindholm et al., 2013). Genotyping experimental mice for the presence of the *t* haplotype (*t* heterozygous, +/*t*) or for its absence (+/+), and afterwards using a carefully designed mating scheme, allowed us to manipulate whether females gave birth to a normally sized or to a smaller litter (for a detailed description of the method see Chapter 5 (Ferrari et al., 2014). This method enabled us to increase the variation in litter size differences between communally nursing females, while remaining in the natural range.

### *Experimental design*

Our experimental treatments comprised females raising their young communally with a full sister (same-aged, familiar littermates) or solitarily as a comparison. Females were on average 89 days old (range 62–209 days) and sexually naive at the beginning of the experiment.

*Communal treatment*

Two full sisters were kept together in a cage system, consisting of three Macrolon Type II cages (18x24 cm and 14 cm high), connected via transparent plastic tubes. An unfamiliar, unrelated male was introduced on day 1 of the experiment. Each social group was kept in the experiment until the two females raised two communal litters together or failed to do so within 100 days.

One of the sisters within a social group ($N$=20 pairs) was +/+, while the other was +/$t$. In 10 social groups the females were kept together with a +/$t$ male; the +/$t$ female was here expected to have a smaller litter than her +/+ sister. In the remaining 10 groups the male was +/+ and we expected no biased difference in litter size between the sisters.

*Solitary treatment*

Two full sisters, one +/$t$ and the other +/+, were each paired monogamously with a +/+ male and kept in two Macrolon Type II cages (18x24 cm and 14 cm high; $N$=21 females; one +/$t$ female failed to give birth). Each social group was kept in the experiment until the female raised two litters.

*Monitoring Reproduction*

Introduction of a genetically unrelated, adult male was considered as day 1 of the experiment. Males were on average 94 days old (range 51–266 days). From day 19 of the experiment onwards, social groups were checked daily for new litters or the number of pups alive from already born litters. Newborn pups in communal nests were individually tattooed (coloured toe tattoo, Aramis Microtattoo Systems) to allow easy discrimination between litters. Pups were considered 1 day of age on the day a litter was first found. In the communal treatment, a tissue sample for genetic analysis was taken of pups found dead, and of pups from litters born on the same day, if they could not be assigned to one of the litters.

*Pup body weight*

We weighed pups to quantify the effect of maternal investment when they were 1 (day of birth), 5, 9, 13, 17, 23 (weaning) and 28 days old, when they were removed from the parental cage. To avoid potential negative influences of the milking procedure (see below) on pup growth, we only used body weight of litters whose females were not milked during that time. In total, 1655 weight measures from 60 litters went into this analysis (38 communally and 22 solitarily reared litters). Not all pups were measured at all ages; exact numbers for each age class can be found in Appendix A Table 13.

*Quantitative milk analysis*

To quantify a female's energy investment during lactation we milked 61 females (40 communally nursing females and 21 solitarily nursing females). Females were milked once when their own pups were 15 days old, which corresponds to the peak of the lactation period (König et al., 1988; König and Markl, 1987). The method used was established by König et al. (1988) and provides a measure for the amount of milk available to pups. The authors showed that the energy delivered via milk corresponded to the energy used by pups for growth and metabolism. Litters within communal nests differed in age (females gave birth on different days). We calculated the average pup age for communal litters on the day of milking by weighting the age of the separate litters with the number of pups each litter contained ((litter size A x age of A) + (litter size B x age of B) divided by the joint litter size of A plus B).

We milked females during their second (49 females), third (10 females) or fourth litter (two females), and all females had successfully raised their own and alien offspring before the milking procedure. Since milk production increases after the first litter and remains rather stable until at least the female's fifth litter (König and Markl, 1987), we thus avoided an effect of primipary on lactation performance or behaviour.

Females were isolated for 3.5 h from their social group before the milking procedure, to minimize the effect of the last suckling event by pups and standardize the amount of milk available. We anaesthetized females with a subcutaneous injection (100 mg of ketamine and 5 mg of xylazine per kg of mouse) in the neck region. To induce milk flow, the already anaesthetized mice were injected with 1 IU (International Unit) of oxytocin intraperitoneally. Each mammary gland was milked with a special milking device developed by Hoffmann et al. (1982) until all available milk was collected. The entire procedure took between 15 and 25 min. For more details see König et al. (1988). The amount of milk was documented in grams (balance: Mettler Toledo, maximum 100 g, d=0.01 mg) and the samples were stored at -20 °C.

To compare the amount of milk females invested in relation to the number of offspring they weaned (number of offspring alive at day 23), we calculated their per capita milk investment, defined as the amount of milk produced by a female, divided by the number of her own weaned offspring. Litter size at weaning was identical to litter size at the time of milking, with one exception, where one pup disappeared on day 16. Communally nursing females were further characterized as whether they reared the smaller or the larger litter in the communal nests.

*Qualitative milk analysis*

We quantified milk quality by analysing the total lipids and total solids from the stored samples. Each milk sample was double tested for both measures, if the available quantity allowed it (each test required 50 mg of milk). The mean between the two replicates was used for later analyses. In total 51 of 61 samples were

analysed (32 samples from communally nursing females and 19 samples from solitarily nursing females). To analyse how much energy females invested in each of their own offspring weaned, we calculated their per capita energy investment. As a proxy for total energy invested we took the amount of lipids (g) a female produced (total amount of milk produced x percentage of lipids in the milk) since lipids represent more than 80% of the energy in house mouse milk (König et al., 1988). We divided that value by the number of the female's own weaned offspring to obtain the amount of energy invested in each of her own offspring (per capita energy investment).

*Total lipids*

Lipid content was analysed gravimetrically with the Röse-Gottlieb method (Baverstock et al., 1976; König et al., 1988). The lipids were dissolved in ammoniac (25%) and then extracted with an ether/petroleum ether mixture.

*Total solids*

Total solids were measured gravimetrically. Milk samples were dried at 102 °C to a constant weight König et al. (1988).

*Statistical Analysis*

Statistical analyses were performed with R Version 3.0.2 (R Core Team, 2015). Generalized linear models (GLM) were conducted, unless the nested design of the study (two sisters together in one social group, several litters per female) required additional random effects to control for dependencies within the data. In these situations linear mixed models (LMM) were performed with the package lme4 (Bates et al., 2014). Fulfilment of model assumptions was inspected visually and the data were transformed if necessary or the appropriate link function was chosen for GLMs.

*Model selection*

Full models were compared to all possible combinations of models containing the same or fewer explanatory factors with the dredge function in the MuMin package (Bartoń, 2014). The best model was determined based on corrected Akaike information criterion (AICc) values. Delta AICc between the best and the second best model had to be at least 2; otherwise two models were considered equal and the one with fewer degrees of freedom was chosen. We used parametric bootstrapping to assess the significance of fixed effects in the most adequate model (for more details see below). Table 1 summarizes for all analyses what type of model we used, the full model and the most adequate model.

Table 1: Full models and most adequate models after model selection are given.

| | | fixed effects: | | random effects: |
|---|---|---|---|---|
| type of model | response variable | full model | most adequate model | |
| **Solitarily nursing females** | | | | |
| GLM (gamma) | milk quantity [g] | gtype+weight+own ls | weight | - |
| LM | milk quality [lipids %] | gtype+weight+own ls | only intercept | - |
| **Communally nursing females** | | | | |
| LMM | milk quantity [g] | weight+own ls+joint ls+pup age | weight+joint ls | group |
| LMM | milk quality [lipids %] | weight+own ls+joint ls+pup age | only intercept | group |
| **Comparison between communally and solitarily nursing females** | | | | |
| LMM | per capita milk investment [g] | weight+ls*trt | weight+trt+ls | group |
| LMM | milk quality [lipids %] | weight+trt*milk produced | trt | group |
| LMM | per capita energy investment [g] | weight+ls*trt | weight+trt+ls | group |
| LMM | pup body weight [g] | (age+ls+lsb+trt+sex)$^{\text{two way interactions}}$ | age*ls+age*trt+age*sex+lsb | group+♀ID/litterID |

List of abbrevations used: gtype= a female's genotype (+/*t* or +/+), own ls= own litter size, joint ls= joint litter size in a communal nest, trt= treatment: solitarily rearing female, dam of the larger litter in a communal nest or dam of the smaller litter in a communal nest, ls= total number of pups in the nest (own litter size for solitarily, and joint litter size for communally nursing females), pup age= average age of pups in communal nests, lsb= litter size at birth, age= pup age[days], group= social group, ♀ID= female identity, litterID= litter identity.

### *Assessing significance of fixed effects in LMMs*

First, we fitted a model without the factor of interest to the observed data and simulated new data using this reduced model. These simulated data were then fitted to the reduced model as well as to the full model that included the factor of interest. By calculating the deviance between the full and the reduced model, we obtained an estimate of how well the full model performed when the factor of interest had no effect. These steps of data simulation and model refitting were iterated 10 000 times. We then used a chi-square test to compare the distribution of differences in deviance to the difference in deviance we observed between the reduced and the full model when fitted to the actual data. To determine which levels of a factor differed from each other, we used, as an alternative to post hoc testing, the confidence interval (CI) of the difference. If the 95% CI of the difference did not cross 0, we assumed two levels to be significantly different from each other.

### *Ethical Note*

All experiments were approved by the Veterinary Office Kanton Zurich, Switzerland (licence no. 65/2011). All females survived the milking procedure and continued to care for pups shortly after they recovered from anaesthesia. No long-lasting negative effect of the milking could be observed. Pup growth was only temporarily reduced for −3 days after milking of a female, and milking did not increase pup mortality. Pups in communal nests were never left without any maternal care, because only one female at a time was milked. In solitary litters pups remained alone before and during milking of their mother for at most 4.5 h. During that time, litters were left at room temperature in the nest built by the mother. Under natural conditions, females leave their pups alone for even longer periods, without negative effects on offspring survival (Auclair et al., 2014a).

## RESULTS

We milked 61 experienced (multiparous) females when their own young were 15 days old to compare the quantity and quality of milk produced by females nursing solitarily versus communally. Litter sizes on that day corresponded to weaning litter sizes in all but one litter (reduction of litter size by one pup between milking and weaning at day 23). Females on average gave birth to 7.1 ± 0.3 pups (range 1–12) and weaned 6.4 ± 0.4 pups (range 1–10; mean ± SE). The number of females milked in the different treatments, as well as summary information about their litters, is given in Table 2.

Table 2: Mean litter sizes at birth and weaning ± SE for solitarily and communally nursing females.

| N | ♀ genotype | ♂genotype | litter size at birth | litter size at weaning | age difference in communal nests |
|---|---|---|---|---|---|
| Communal treatment | | | | | |
| 10 | +/+ | +/+ | 8.5±0.8 | 7.0±0.8 | 4.2±1.3 |
| 10 | +/*t* | | 7.5±0.9 | 6.5±0.9 | |
| 10 | +/+ | +/t | 6.9±1.0 | 6.1±1.0 | 4.7±1.2 |
| 10 | +/*t* | | 3.7±0.5 | 3.1±0.5 | |
| Solitary treatment | | | | | |
| 11 | +/+ | +/+ | 7.8±0.4 | 7.5±0.5 | |
| 10 | +/*t* | +/+ | 8.0±0.6 | 7.9±0.5 | |

In agreement with the results of König et al. (1988), we found a significant and positive correlation between pup body weight gain from day 1 (birth) to day 13 and the amount of milk obtained from their mother at day 15, for both male and female pups (Spearman correlations: females: rho=0.70, $N$=15, $P$=0.005; males: rho=0.62, $N$=15, $P$=0.015; Fig. 1).

### *Regulation of milk production in lactating females*

We first assessed for solitarily and communally nursing females the influence of female genotype, body weight and litter size on the amount and quality of milk produced. We did so separately since analysis of communal nursing required the incorporation of both the female's own litter size and the number of pups in the joint litter.

### *Milk quantity and quality of solitarily nursing females*

Females rearing their young alone nursed litters ranging in size from four to 10 pups. The average litter size at weaning was 7.7 ± 0.4 pups. Heavier females produced significantly more milk than lighter females when their own young were 15 days old ($\chi^2_1$=15.08, $P$<0.001, $N$=21; Fig. 2).

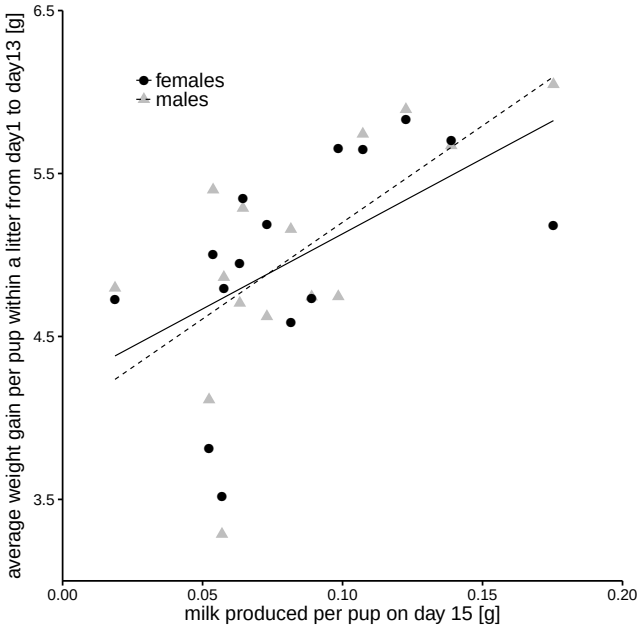


Figure 1: Average weight gain of solitarily reared male and female pups between day 1 (birth) and day 13 as a function of the amount of milk collected from their mother at day 15 ($N$= average weight gain from 15 litters for both male and female pups).

The amount of milk produced was not significantly influenced by a female's litter size ($\chi^2_1$=0.26, $P$=0.609, $N$=21) or her genotype (+/+ or +/$t$; $\chi^2_1$=0.20, $P$=0.654, $N$=21).

Milk quality, the percentage of total lipids in the milk, was not significantly influenced by the female's own litter size, body weight or genotype. The most adequate model only contained the intercept. We found no significant difference between +/$t$ and +/+ females in milk quantity or quality. For the rest of the analyses, we therefore omitted this factor.

*Milk quantity and quality of communally nursing females*

Females rearing their young communally nursed joint litters from three to 19 pups. The average joint litter size at the time of milking was 11.4 ± 0.7 pups (mean ± SE). The average litter size at weaning (the female's own offspring) for communally nursing females was 5.7 ± 0.5 (range 1–10). The two litters of a joint communal nest differed on average by 3.5 ± 0.5 pups at the time of milking, and females sharing a communal nest differed similarly in the number of pups weaned. There was no significant difference between sister pairs mated to +/+ or +/$t$ males in terms of absolute litter size difference (Wilcoxon rank sum test: W=44.5, $P$=0.70), but it was the +/$t$ female that had the smaller litter in the latter groups, as expected based on the recessive lethality of the $t$ haplotype.

Communally nursing females produced more milk with increasing joint litter size (parametric bootstrapping,: $\chi^2$=7.59, $P$=0.010, $N$=40; see Table 3, Fig. 3a). Simi-

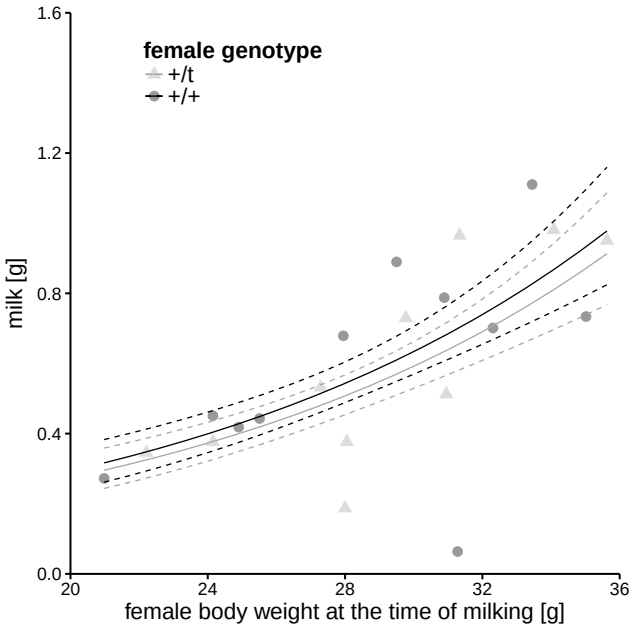


Figure 2: Milk (g) collected from solitarily nursing females after having been separated from their young for 3.5 h as a function of female body weight at the time of milking; plotted separately for two genotypes ($N$=11 +/+ and 10 +/$t$ females). Model estimates (mean) and the SE of the mean are shown ($N$=21 females).

lar to solitarily nursing females, milk production increased with increasing female body weight at the time of milking (parametric bootstrapping: $\chi^2$=6.16, $P$=0.020, $N$=40; see Table 3, Fig. 3b). Neither a female's own litter size nor the average age of the joint litter had a significant effect on the amount of milk produced. Neither a female's body weight nor her own or the joint litter size had a significant effect on her milk quality (percentage of total lipids and total solids) at peak lactation (when her own pups were 15 days old). The best model in both instances only contained the intercept.

*Comparison of solitarily and communally nursing females*

To analyse whether females use different investment strategies during solitary versus communal nursing, we compared the mothers' milk quality and their milk and energy investment per their own pup weaned.

*Differences in milk quality*

Total solids and total lipids were highly positively correlated (Spearman correlation test: rho=0.72, $N$=51, $P$<0.001). Most of the energy provided to pups in the milk is in the form of lipids (König et al., 1988). We therefore used only total lipids for further analyses.

The percentage of total lipids in the milk ranged from 12% to 29.1%. Overall, milk of communally nursing females contained significantly fewer lipids than milk

Table 3: Factors influencing the amount of milk produced by communally nursing females after a 3.5 hour separation from the litters. Displayed are model estimates [mean](LMM) and 95% confidence intervals. The final model had the following fixed effect structure: milk [g] ~ female body weight at the time of milking + total number of pups in the nest ($N$=40 females within 20 social groups). The communal nest was used as random factor to correct for independencies in the data.

| factor | model estimates | 95% CI |
|---|---|---|
| body weight [slope] | 0.03 | 0.01–0.05 |
| joint litter size [slope] | 0.03 | 0.01–0.06 |

of solitarily nursing females (parametric bootstrapping: $\chi^2$= 11.15, $P$=0.007; see Fig. 4a). There was no significant difference between sisters sharing a communal nest; mothers of the smaller litters in communal nests produced milk of similar lipid content as mothers of the larger litters. The total amount of milk a female produced, her body weight at the time of milking and the total litter size (joint litter size for communal and their own litter size for solitary females) had no significant effect on the percentage of lipids in the milk.

*Per capita milk investment*

We analysed how much milk communally and solitarily nursing females produced per their own weaned offspring (per capita milk investment) after a 3.5 h long separation from the nest to test for differences in the investment of solitarily and communally nursing females. The amount of milk produced ranged from 0.0006 g to 0.4 g per their own weaned pup. Communally nursing females were further divided into mothers of the smaller or larger litter within a communal nest. The three classes of females differed significantly in the amount of milk they produced per their own weaned offspring ($\chi^2$=13.84, $P$=0.002; see Fig. 4a). More precisely, the mother of the smaller litter in a communal nest had a higher per capita milk investment than her partner with the larger litter (model estimate of the difference (confidence interval of the difference) between dams of smaller and larger litters in communal nests: 0.05 g milk per their own weaned offspring: 0.01–0.09). The mother of the smaller litter also produced significantly more milk per her own weaned offspring than a solitarily nursing female (0.08 g, 0.04–0.12). Solitarily nursing females did not differ significantly in per capita milk investment from a mother with the larger litter in a communal nest (0.03 g, -0.01–0.07). The larger the joint number of pups in the nest, the lower was the per capita milk investment (parametric bootstrapping: $\chi^2$=6.98, $P$=0.022). Heavier females gave more milk per their own offspring (parametric bootstrapping: $\chi^2$=7.56, $P$=0.009).

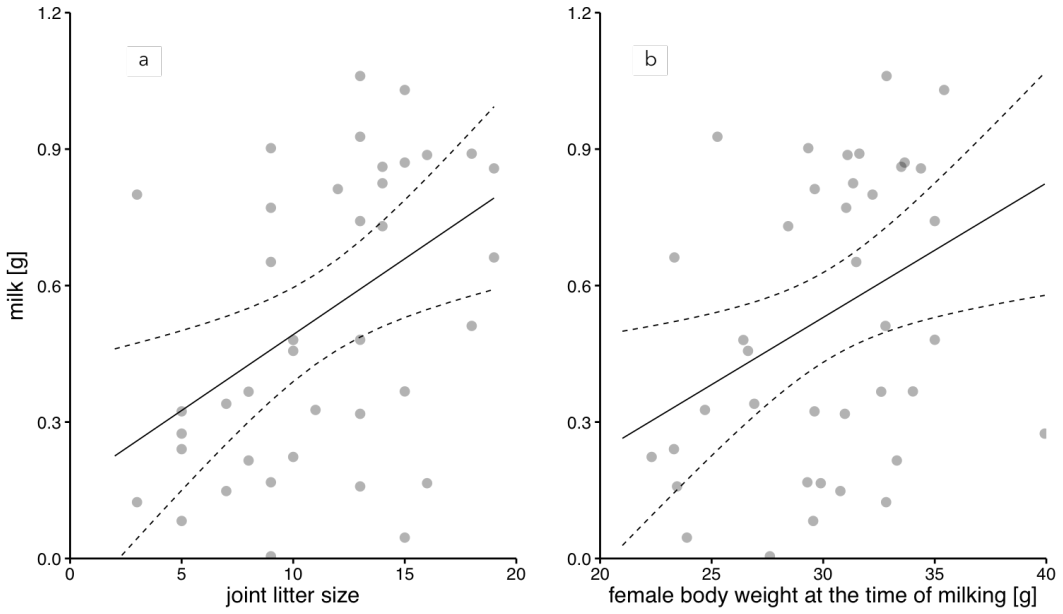


Figure 3: Milk (g) produced by communally nursing females after having been separated from their young for 3.5 h as a function of (a) the joint litter size in the communal nest (corrected for female body weight) and (b) female body weight at the time of milking (corrected for litter size). Model estimates (mean) and the 95% confidence interval of the mean are shown ($N$=40 females).

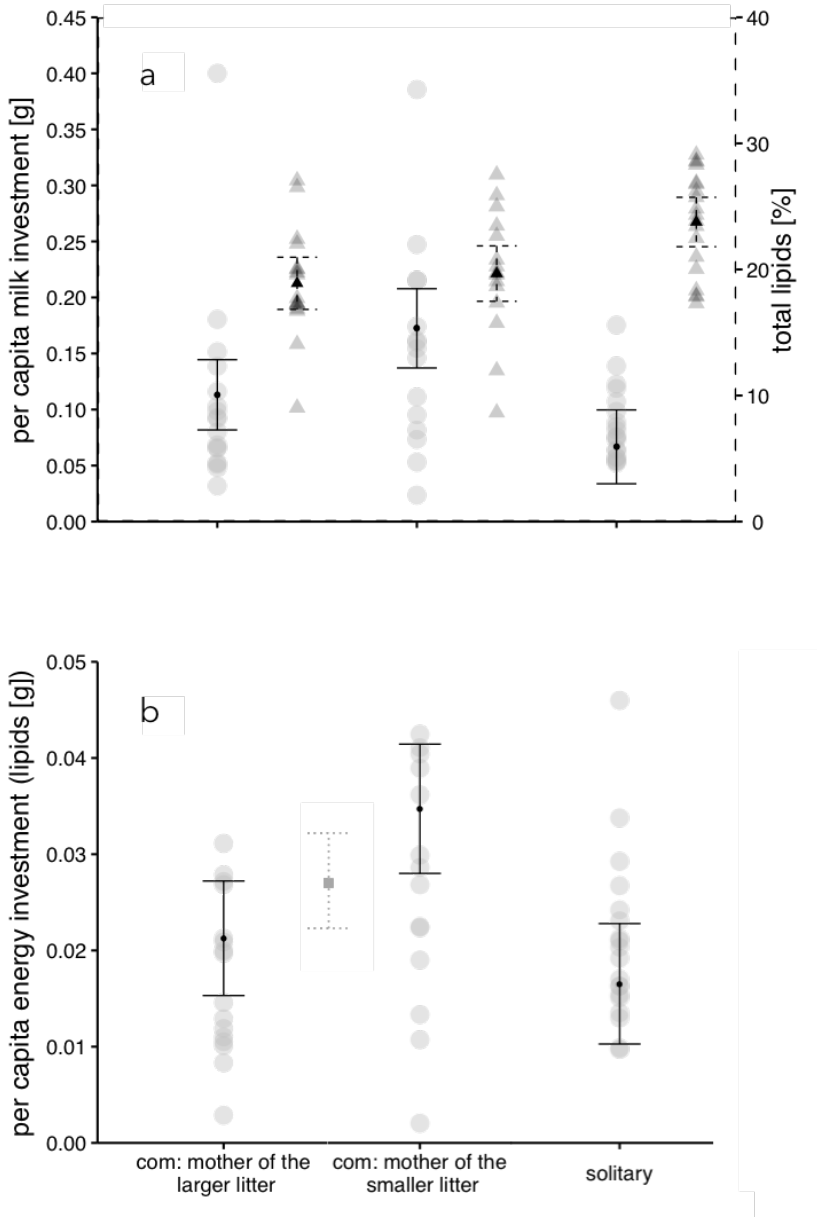


Figure 4: (a) The amount of milk produced per a female's own weaned offspring (per capita milk investment, circles) and the milk's lipid content (%) (triangles) for communally (com; dams of smaller versus larger litters within communal nests) and solitarily nursing females, measured as the amount of milk collected from females after having been separated from their young for 3.5 h. (b) Lipids (g) produced per a female's own weaned offspring (per capita energy investment) by communally (com; dams of smaller and larger litters within communal nests) and solitarily nursing females. The overall mean for communally nursing females is indicated in grey lines. Model estimates (mean) and the 95% confidence interval of the mean are shown ($N$= 51 females; 32 communally nursing females and 19 solitarily nursing females).

*Per capita energy investment*

In a last step, we analysed how much energy (measured as grams of lipid) communally and solitarily nursing females produced per their own weaned offspring (per capita energy investment). Communally nursing females were again further classified as the mother of the smaller or the larger litter within a joint nest. The energy females invested per their own offspring differed significantly between the three classes of females, as was expected based on the results from the per capita milk production and the lipid content analysis ($\chi^2$=13.27, $P$=0.002; see Fig. 4b). The mother of the smaller litter in a communal nest invested the most energy per her own weaned offspring, significantly more than the mother of the larger litter (model estimate of the difference (confidence interval of the difference): 0.01 g, 0.005–0.022) or than solitarily nursing females (0.02 g, 0.008–0.028). There was no significant difference between dams of larger litters in communal nests and solitarily nursing females. With increasing number of pups in the joint litter, females decreased their per capita energy investment (parametric bootstrapping: $\chi^2$=12.41, $P$=0.001). The latter increased, however, with increasing female body weight (parametric bootstrapping: $\chi^2$=12.41, $P$=0.001).

*Growth rates of pups reared communally or solitarily*

Pups raised in communal nests grew faster than solitarily reared pups (significant interaction term: $\chi^2$=285.3, $P$<0.001; see Fig. 5). The larger the litter (for solitary nests) or the joint litter (for communal nests), the less steep was the growth curve (significant interaction term: $\chi^2$=98.7, $P$<0.001). Additionally, we found a significant sex difference with males becoming heavier than females with increasing age (significant interaction term: $\chi^2$=42.9, $P$<0.001; see Fig. 5). There was a nonsignificant trend for smaller pup body weight with increasing birth litter size ($\chi^2$=3.0, $P$=0.10).

## DISCUSSION

Our results on milk investment during peak lactation revealed that communally nursing females cooperated while rearing litters in a communal nest. They shared the costs of lactation by investing according to the combined number of their own and alien offspring in the nest and not their own litter size. This suggests that females indeed indiscriminately nursed their own and alien young. At the same time, our results showed that cooperating females did not benefit equally when they differed in litter size. Although they shared the costs of milk production, the payoff, i.e. the number of weaned offspring, differed for the two cooperating partners.

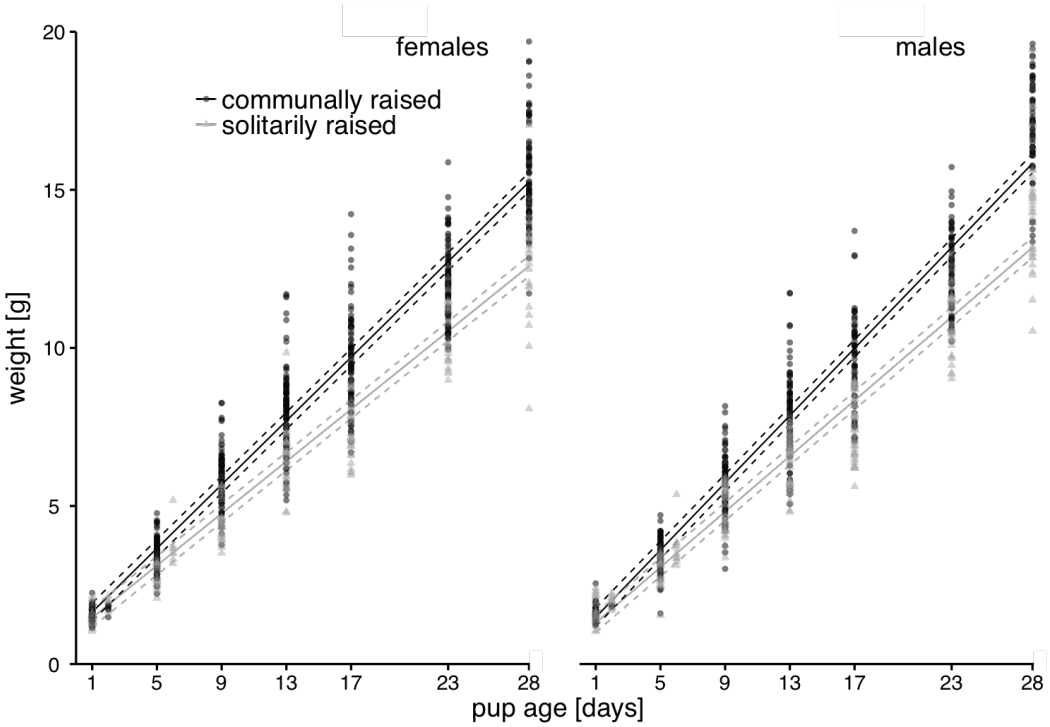


Figure 5: Body weight of (a) female and (b) male pups raised in communal or solitary nests. Pup body weight was taken at day 1 (birth), 5, 9, 13, 17, 23 (weaning) and 28 (removal from the parental cage). Model estimates (mean) and the 95% confidence interval are shown ($N$=1655 weight measures from 60 litters; of which 38 were raised communally and 22 solitarily; for sample sizes of pups for all age classes and both treatments see Appendix A Table 13.

*Regulation of milk production in lactating females*

Lactation performance of solitarily and communally nursing females was significantly influenced by a female's body weight at the time of milking. Heavier females, likely to be in better condition, gave more milk as has been described before (Knight et al., 1986). There was, however, large variation in the amount of milk females produced. The causes of this variation are unclear. Females might vary in their condition, and therefore overall milk production, or vary in their response to the oxytocin administered to induce milk release. Another source could be that varying amounts of milk might still have been present in a female's mammary glands at the time of separation from their young (3.5 h before the milking) As we do not expect a systematic bias in our data, we none the less used the amount and quality of milk collected after a period of 3.5 h of separation from the pups as an estimate of a female's milk production.

We failed to show an effect of a female's own litter size on absolute milk production in solitarily nursing mice. This finding is in contrast to previous studies conducted on house mice exclusively nursing their own litters (Knight et al., 1986; König et al., 1988). However, in communal nests, we found a significant increase

in milk production in communally nursing females with increasing total litter size (their own and alien offspring). In communal nests, the range in total litter size was larger than in solitary nests. The per capita milk and energy investment analyses, which were performed for solitarily and communally nursing females together, revealed a significant effect of the number of pups that was similar for the two treatments (no significant interaction). Thus, we assume that our small sample size for the solitary treatment (N=21), given the large variation in milk production, together with the small variation in litter size among those females, can explain why we did not find an effect of litter size on the amount of milk produced at peak lactation among solitarily nursing females.

There is evidence that female house mice are able to adjust their investment to postpartum changes in litter size (Knight, 1982; König et al., 1988). One potential mechanism allowing adjustment in milk production is the suckling stimulus. Suckling by pups maintains lactation for several weeks in rats (Bruce, 1961) and milk production in mice correlates positively with the number of suckling young (Bateman, 1957). Similar results were found for other mammalian species (Alexander and Davies, 1959; Hayden et al., 1979) and suckling also plays a role in regulating a female's food intake during lactation (Cotes and Cross, 1954). An inability on the female's side to fend off or discriminate against alien offspring, and as a consequence the joint litter suckling, could therefore explain the effect that communally nursing females increased milk production with increasing joint litter size. Furthermore, such a mechanism would also explain why females in communal nests tended to produce more milk per their own weaned offspring than their solitarily nursing conspecifics. Communal litters are larger than solitary ones and females as a consequence are exposed to more suckling young, given that females sharing a nest usually do not nurse simultaneously (Auclair et al., 2014a).

*Differential investment of communally and solitarily nursing females*

Milk quantity is not the only factor that determines how much energy a female invests in her offspring. Milk varies extensively in quality and females may, instead of adjusting the amount of milk they produce, alter its quality. Such an adjustment would seem especially beneficial if females are not able to discriminate against alien offspring and therefore cannot elude the increased suckling stimulus.

Our results showed that while communally nursing females overall produced more milk in relation to their own litter size than solitarily nursing females (per capita milk investment), their milk was of lower quality since it contained fewer lipids (see Fig. 4a). It has been described before that mammals can adjust their milk quality, in the context of differential sex allocation, with females producing richer milk when nursing sons (Landete-Castillejos et al., 2005; Hinde, 2007).

This lower milk quality might reflect a constraint if females are not able to produce large amounts of milk of high lipid concentration. However, we did not observe a reduction in milk lipids with increasing amount of milk produced in communally nursing females. Alternatively, we suggest that the relatively low lipid concentration serves as a mechanism to minimize overinvestment. Communally

nursing females increased milk production with increasing number of suckling pups. They counteracted that enlarged milk investment, however, by producing milk of lower energy content in comparison to solitarily nursing conspecifics. Such a strategy might reduce the potential costs of being exploited by generally lowering maternal investment. This finding is in agreement with Hager and Johnstone (2007), who found that females rearing mixed litters (their own and cross-fostered alien pups) provided fewer resources to the litter (indirectly measured over pup growth), than females only rearing their own young. In an analogy to the biparental care situation, our results might also reflect a lower overall investment when females negotiate over the amount of maternal care they provide to the offspring (Lessells and McNamara, 2012). Nevertheless, communally nursing females on average still invested more energy (grams of lipids) per their own weaned offspring than solitarily nursing females. This higher investment resulted in higher weaning weight in pups of communally nursing females, as has been found previously (Sayler and Salmon, 1969). Pups in communal nests might further benefit from shorter intervals between nursing bouts if females take turns nursing their offspring, allowing for more efficient growth. While communal nursing in our experiment did not allow females to reduce the costs of milk production (higher amount of lipids produced per their own weaned offspring than solitarily nursing females), their young were heavier at weaning and as a consequence probably in better condition, which could promote their survival and success.

*Communally nursing females do not benefit equally from cooperation*

The observed reduction in milk quality in communally nursing females did not serve as a mechanism to prevent one female from being exploited. Dams of both the larger and the smaller litter produced milk of similar quality in terms of the percentage of milk lipids. As a consequence, females with smaller litters than their social partner overinvested in relation to their own litter size (see Fig. 4). They produced more milk (per capita milk investment) and invested more energy (per capita energy investment) per their own offspring than both dams of larger litters in communal nests and solitarily nursing females. The overproduction may seem small, but the additional costs could influence a female's future reproduction. Fuchs (1982) showed that the interval between the first and second litter of a female house mouse increases with increasing number of pups in the first litter (coinciding with an increase in the amount of milk produced during rearing of the first litter). Female bank voles, *Myodes glareolus*, that nursed larger litters had a lower survival probability and tended to give birth to a smaller subsequent litter (Koivula et al., 2003). The dams of the larger litters in our experiment, on the other hand, benefited by weaning heavier offspring with a similar per capita energy investment to solitarily nursing females.

Differences in litter size are common among communally nursing females. We experimentally increased the variance in litter size in half of the groups in our communal treatment. The actual differences in litter size at the time of milking were nevertheless similar to those in the unmanipulated groups. Under natural

conditions, as well as in our laboratory setting, differences in litter size are often caused by female infanticide in communal nests (König, 1994a; Palanza et al., 2005; Schmidt et al., 2015). We therefore expect varying benefits for communally nursing females also to occur under natural conditions and not just be a by-product of our manipulation.

Females in the wild might alter their investment by spending more or less time nursing the communal litter, depending on their contribution. In a wild population, however, the time a female spent in the nest was unaffected by the number of her own pups in the communal nest (Auclair et al., 2014a), suggesting that females did not behaviourally adjust their investment to their own litter size. Time spent in the nest, or even time spent nursing, might not be an ideal proxy for female investment (Cameron, 1998), but it is usually the only source of data available in wild populations.

We used familiar full sisters for our experiments, which could have caused the high levels of exploitation observed. A high degree of relatedness between cooperating partners reduces the costs of being exploited for the female of the smaller litter in comparison to being exploited by an unrelated individual, through indirect fitness benefits. At the same time, however, it reduces the benefits of the exploiting female through a loss in indirect fitness (Mathot and Giraldeau, 2010). Theoretical and empirical work shows that higher levels of cheating and exploitation will be tolerated among relatives whenever the exploited individual has a certain level of control (Mathot and Giraldeau, 2010). If a lactating female that is joined and exploited by a relative is more likely to stay and overinvest instead of abandoning the communal litter than one exploited by an unrelated partner, we expect a higher asymmetry among related than unrelated females. Further studies are needed to test whether the observed asymmetry is indeed a consequence of the female's relatedness in our experiment.

Females in our experiment had no opportunity to choose a social partner. In addition, confinement within the cages might not have allowed them to raise their young solitarily, if they preferred to do so. Our observation that females invested according to the total number of pups in a communal nest with different litter sizes might have been a side-effect of such constraints imposed by laboratory conditions. Under natural conditions, female house mice may use social partner choice (see above) or the decision to nurse solitarily as a mechanism to avoid exploitation. In such a case we do not expect strategies to evolve that avoid exploitation after a communal nest is formed. Our results support this hypothesis. We suggest that the apparent inability of females to discriminate their own from alien young resulted in females producing milk according to the total litter size in the nest. At the same time, this inability might reflect an evolutionary constraint forcing females to resolve the conflict prior to the formation of the communal nest, for example by means of social partner choice.

A mother's inability to recognize her own offspring might be in her pups' interest and may represent an outcome of parent-offspring conflict, in favour of the offspring. Alternatively, male imprinting has been suggested to explain this phenomenon Roulin and Hager (2003). A male mating with both females sharing a

nest has an interest in equal investment in all of his offspring, irrespective of the female's share. However, wild house mice are polygynandrous and males very rarely have exclusive paternity of offspring in a communal nest (Auclair et al., 2014b). Currently, it is not known what mechanism prevents lactating female house mice from recognizing their own offspring or expressing offspring recognition.

*Conclusions*

Cooperating females faced a situation of conflict during communal nursing as soon as they differed in litter size. Since females invested according to the joint and not their own litter size, an asymmetry in the benefit of cooperation (the number of weaned offspring) resulted. Such a conflict among cooperating partners is characteristic of a public good. A public good is a resource used or a collective good produced by several individuals that benefits the whole group (Rankin et al., 2007). Group members have an incentive to cheat by overexploiting the good, because the costs are shared among all individuals, leading to the collapse of the public good (Rankin et al., 2007). Indiscriminate parental care in a communal nest results in the costs being shared by all investing females, but those with more offspring in the joint litter or clutch will benefit more. What mechanisms prevent the collapse of the public good in such a situation? How can females prevent their social partners from lowering their investment, or even abandoning the joint litter, resulting in intraspecific nest parasitism?

In a laboratory study, female wild house mice that nursed their litters communally with a sister had a higher lifetime reproductive success than both solitarily nursing females and females that communally nursed their young with an unrelated individual, and females cooperated repeatedly (König, 1994a). Short-term disadvantages in one cooperative event (being the dam of the smaller litter) might thus be outweighed if the female contributes the larger litter in the following communal nest. Data over a longer period, or from a wild population, could help to shed light on these questions and determine the actual costs and benefits of rearing offspring communally. Additionally, females may benefit from choosing a nursing partner carefully. Females should preferentially cooperate with a partner that has a similar litter size to reduce the potential for conflict. If the female with the smaller litter has control over who is exploiting her, we would also expect communal nursing to be more common among relatives, as discussed above. In a recent study in a wild population, Weidt et al. (2014) found evidence that females do not always nurse communally when given the choice, and that the number of available partners influences a female's propensity to nurse communally, indicating that choice indeed plays a role. We conclude that the potential for conflict among communally breeding species that indiscriminately nurse or feed all young in the nest may be high, when expected contributions to the joint clutch or litter are not random. Here we showed that in house mice the benefits among communally nursing females could even vary up to the point where one female is in fact exploited by her social partner.

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# CHAPTER II

# 2

# A REDUCED PROPENSITY TO COOPERATE UNDER ENHANCED EXPLOITATION RISK IN A SOCIAL MAMMAL

Manuela Ferrari, Anna K. Lindholm, Barbara König

## ABSTRACT

Conditional adjustment of cooperativeness to the expected pay-off might be a useful strategy to avoid being exploited in public good situations. Parental care provided towards all offspring in a communal nest (containing offspring of several females) resembles a public good. Females indiscriminately caring for all young share the costs equally, but the pay-off may vary depending on their contribution to the joint nest (number of own offspring). Females with fewer offspring in the joint nest will be exploited and overinvest relative to their contribution. We experimentally created a situation of high conflict in communally nursing house mice, by using a genetic tool to create a difference in birth litter sizes. Females in the high conflict situation (unequal litter sizes at birth) showed a reduced propensity to give birth as part of a communal nest, therefore adjusting their cooperativeness to the circumstances.

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KEYWORDS: communal nursing, cooperation, house mouse, lactation, public good, reproductive conflict

## INTRODUCTION

The use of public resources leads to conflict, known as the collective action problem (Olson, 1965) or the public goods dilemma (tragedy of the commons) (Hardin, 1968). Individuals have an incentive to increase their own benefit by cheating at the expense of the other group members. Many examples of cooperation in animals can be classified as such social dilemmas and raise the question of how cooperation is stabilised (Rankin et al., 2007). Both theoretical and empirical research revealed that kin selection or coercion (punishment) can prevent the collapse of the cooperation in a public goods dilemma (van Dijk et al., 2014; Peters et al., 2004; Kümmerli et al., 2009; Wenseleers et al., 2004; Fehr and Gächter, 2000). However, the importance of punishment in animal systems is debated (Raihani et al., 2012).

The classical public good is defined as non-excludable, meaning that individuals cannot be excluded from the benefits. However, situations exist in which individuals have the option to be neutral bystanders and not join a cooperation. Under such conditional cooperation individuals decide based on the context whether to participate in the cooperation or not. This opens the possibilities for other mecha-

nisms to stabilise cooperation. In a theoretical model Hauert et al. (2007) showed for example that the evolution of costly punishment is facilitated under such conditional cooperation.

Cooperative offspring care is a situation that resembles a public good and may be conditional. Communal breeding or joint nesting can be observed in many different taxa (social spiders (Schneider, 2002), insects (Heg et al., 2006), birds (Mumme et al., 1988) and mammals (König, 1997)). In communally caring species several females pool their clutches or litters in one nest or help raise the offspring of others, with varying degrees of reproductive skew among females. The public good is the parental care provided by the females (and potentially also by males or non-reproducing helpers) towards all young. In species where several reproducing females indiscriminately care for offspring in a joint nest, females share costs equally but the benefits for the individuals can vary depending on the number of offspring they have in the nest and the amount of care they provide. Indiscriminate care of young has been described for a number of communally breeding species such as as beetles (Eggert and Müller, 1992), birds (Riehl and Strong, 2015), bats (Watkins and Shump, 1981) and rodents (Holmes and Sherman, 1982). Communal offspring care may also be conditional since individuals can choose to participate in the public good (by forming a communal nest) (McShea and Madison, 1984; Scott and Williams, 1993; Lott and Mastrup, 1999), or nest solitarily instead.

This is the case for house mice (*Mus musculus domesticus*), a species in which females show two different breeding strategies, rearing their young either solitarily, or communally together with one or several other females (Auclair et al. (2014a) observed on average 2.2 $\pm$ 0.1 [mean $\pm$ SE] females per communal nest). A recent field study revealed that females did not always communally nurse when given the opportunity (when at least one other female in the social group had dependent offspring at the time the focal female gave birth). Only 33% of those females formed communal nests; the other 67% raised their young solitarily instead (Weidt et al., 2014). Such a low percentage seems surprising, considering that in a laboratory setting, females nursing their young communally together with a sister were found to have an increased lifetime reproductive success in comparison to solitary nursing females (König, 1994a). Further benefits described for communal nursing in mice are an increased pup survival (Manning et al., 1995; Auclair et al., 2014b) and a reduction in the time females allocate to spending with their young, without increasing the total amount of time pups were alone (Auclair et al., 2014a). The relative low frequency of communal nursing indicates that females might not always benefit from cooperation. Analysing the potential for conflict among females and under what conditions they decide against cooperation could help to understand the mechanisms stabilising it.

Females rearing litters communally do not discriminate between their own and alien offspring (König, 1989a) and produce milk according to the total number of pups in the joint nest and not their own litter size (Ferrari et al., 2015), which provides scope for exploitation. As soon as females differ in litter size, the one with the larger litter will exploit the other. Litter size differences arise if females give birth to differently sized litters, or if litters differ in their survival probability

after birth. We would expect the conflict potential to be the smallest and females most likely to cooperate when they benefit equally, in other words, if they have similar litter sizes, and to be less cooperative when litters differ in size.

One way to minimise exploitation and as a consequence the collapse of the public good is to decide against communal nursing in an enhanced conflict situation, when litters differ in size. This would require females to have information on not only their own, but also the litter size of their potential social partner, enabling them to adjust their propensity to cooperate to the circumstances. According to this hypothesis, females with the smaller litter size will not form communal nests and therefore avoid the public good situation if there is a pronounced asymmetry in the expected pay-off.

Alternatively females may reduce the conflict by adjusting their partner's litter size through infanticide. Infanticide towards pups that are not their own has been described for female house mice and other mammals and birds, with females giving birth (or laying eggs) first being more susceptible to infanticide (Andersson and Eriksson, 1982; Koenig et al., 1995; König, 1994a). Considering that females are unable to discriminate between own and alien offspring, they should only be infanticidal while still pregnant. In addition, we expect female infanticide to be constrained by the partner's interest. If a female kills too many pups, the partner might leave the empty nest or small litter, before the second female gives birth, because the costs of abandoning the litter may be smaller than staying and raising almost exclusively another female's litter. Under this hypothesis we predict female induced infanticide to correlate with the difference in litter size, with the second female killing more pups if her partner has a larger litter than herself. To test the two hypotheses, we experimentally created asymmetries in litter sizes of two familiar full-sisters within a social group to analyse their behaviour and their propensity to engage in communal nursing. We used a genetic tool to prenatally manipulate litter sizes, which allowed us to (i) measure a female's propensity to cooperate under an enhanced conflict situation and (ii) test whether female infanticide serves as a tool to minimise conflict by equalising litter sizes.

## MATERIAL AND METHODS

### *Animals and Husbandry*

Laboratory born F1 to F3 descendants from a wild house mouse population near Illnau, Switzerland, were used as study subjects. For a description of the wild population of origin see König and Lindholm (2012). The experiments were conducted in Zurich between April 2011 and December 2012. Mice were kept under a constant light:dark cycle of 14:10 hours (light on at 5:30 hours CET) and at a temperature of 22-24°C. Food (laboratory animal diet for mice and rats, no 3430, Kliba) and water were provided *ad libitum*, along with paper towels and cardboard that served as nest building material.

*Experimental Design*

Females were on average 93 days old (range: 62–209 days) and sexually naive at the beginning of the experiment. Two full sisters (litter mates) were kept together with an unrelated male in a cage system, consisting of three Macrolon Type II cages (18x24x14 cm), connected via transparent plastic tubes. Such a set-up was used in previous studies and may allow females to defend a cage each (Weidt et al., 2007). A pair of females living together with a male in a cage system from here onwards will be referred to as a social group.

To manipulate litter size and create an asymmetry between females in our experimental groups, we used the *t* haplotype, a selfish genetic element carrying a lethal allele, present in our wild population of origin (see Appendix B or Chapter 5 (Ferrari et al., 2014)). Within each group, one of the two sisters was +/+, while the other was +/*t*. Using +/*t* and +/+ females in both the experimental and control treatment allowed us to control for potential effects of the *t* on female behaviour. In the experimental treatment (n=14 pairs, 28 females) females were kept together with a previously unfamiliar, genetically unrelated +/*t* male. The +/*t* female was therefore expected to have a smaller litter than her +/+ sister, due to *in utero* mortality of *t*/*t* homozygous embryos (Lindholm et al., 2013). In the control treatment (n=11 pairs, 22 females) the male was +/+ , and as a consequence no substantial differences in litter size between females were expected. Males remained with the females for the whole duration of the experiment. The experiment was stopped as soon as females had raised two communal nests, or if the females did not raise two communal nests within 100 days.

40 out of 50 females were milked while raising their last litter as part of a different experiment (Ferrari et al., 2015). Milking was shown to have no effect on offspring survival probability and those litters were therefore included in analyses here.

*Monitoring Reproduction*

From day 19 after introduction of the male, we checked social groups daily in the morning for newborn pups and documented the total number of pups. We did not handle the pregnant females to avoid the risk of stress induced abortions. Tissue samples were taken from pups found dead, as well as from pups alive at weaning to assess their maternity if they could not be assigned to one of the two litters (see Appendix B for more information about the genotyping and parentage analysis). Litters were removed from the group when 28 days old.

Mouse pups start to forage independently when they are 17 days old and are fully weaned with 23 days. Following König (1994a) we defined a communal nest as two litters being born within 17 days of each other and being raised in one nest. When litters were more than 17 days apart in age, we did not consider this as a communal nest because the older litter was no longer fully dependent on milk and had only a small influence on female investment.

Table 4: Summary of statistical models used for data analyses. Full models and most adequate models after model selection are given.

| type of model | response variable | fixed effects: full model | most adequate model | random effects: |
|---|---|---|---|---|
| **Effect of the *t* haplotype on litter size** | | | | |
| LMM | litter size at birth | ♂genotype*♀genotype | ♂genotype*♀genotype | social group/ ♀ID |
| LMM | litter size at weaning | ♂genotype*♀genotype | ♂genotype*♀genotype | social group/ ♀ID |
| **Propensity to engage in communal nursing** | | | | |
| GLM (quasibinomial) | proportion succ. communal nests | trt | only the intercept | – |
| GLM (quasibinomial) | proportion of communal nests | trt | trt | – |
| **Infanticide** | | | | |
| GLMM (binomial) | proportion of pups alive (weaning) | trt*♀genotype*ord+agediff | ord | cnID[c] /♀ID |
| GLMM (poisson) | # pups killed[a] | ls diff+litter size at birth | –[b] | social group/♀ID |
| GLMM (binary) | birthing order (1 or 2) | trt*genotype+wstart+wbirth+ls diff | only the intercept | cnID[c] / ♀ID |

List of abbrevations used: ♂ or ♀ genotype= male or female genotype (+/*t* or +/+); trt= treatment (control or experiment), proportion succ. communal nests= proportion of successful communal nests (at least one pup from each litter survived to weaning) in relation to all communal nests (successful and unsuccessful ones); proportion of communal nests: proportion of communal nests in relation to all reproductive events (communal nests and solitary nests); ord= birth order within the nest (first or second born litter); agediff= age difference between the litters $[\sqrt{(\text{age of focal litter} - \text{age of other litter})^2}]$; ls diff= difference in litter size between the females; cnID= communal nest identity; ♀ID= female identity; litterID= litter identity; wstart: female body weight at the beginning of the experiment; wbirth: female bodyweight after having given birth. a) Only first born litters were used for this analysis. b) No AICc can be calculated for a GLM with a poisson error distribution and all factors were retained in the model for further analyses. c) In those two analysis the cnID was used as random factor instead of the social group, because the two litters within a communal nest were directly compared to each other specifically.

### *Statistical Analysis*

All statistical analyses were performed with R Version 3.0.2 (R Core Team, 2015). Generalised linear models (GLM) were conducted, unless the nested design of the study (two sisters within a social group, several litters per female) required additional random effects to control for dependencies within the data. In these situations linear (LMM) and generalised linear mixed models (GLMM) were performed with the package lme4 (Bates et al., 2014). Fulfilment of model assumptions was inspected visually and the data were transformed if necessary or the appropriate link function was chosen. GLMs and GLMMs with a binomial error distribution were tested for overdispersion.

During model selection the full model was used as starting model and then compared against all lower level models. Models were ranked based on their AICc value and the one with the lowest value chosen as best model. If two or more models were within 2 delta AICc of each other, the one with the lower number of degrees of freedom was used. Table 4 summarises all analyses, giving the type of model used, the full model and the most adequate model after model selection. To assess the significance of fixed effects parametric bootstrapping was used (see Appendix B for more details).

## RESULTS

### *Effect of the t Haplotype on Litter Size*

Pairing +/*t* males with +/*t* females resulted in litter size reduction at birth in the experimental treatment (mean [95%CI] of the difference in the number of pups between +/*t* and +/+ females, 2.5 [1.6–3.5]) compared to the control (0.2 [-1.0–1.3]), due to the recessive lethal nature of the *t* haplotype (LMM: parametric bootstrapping, ♂genotype / ♀genotype interaction: $\chi^2$=8.82, P=0.004, see Fig. 6 A). Our treatment hence succeeded in creating a situation of enhanced conflict among females based on different birth litter sizes.

+/+ females gave birth to (mean [95%CI]), 7.2 [6.0–8.3] pups per litter in the control treatment (paired with a +/+ male) and 6.1 [5.1–7.1] pups in the experimental treatment (paired with a +/*t* male). +/*t* females had on average 7.0 [5.9–8.2] pups in the control treatment and 3.5 [2.5–4.5] in the experimental treatment (Fig. 6 A). Litter size differences at weaning between +/+ and +/*t* females were not significant, but tended to be larger in the experimental than the control treatment (LMM: parametric bootstrapping: ♂genotype /♀genotype interaction: $\chi^2$=4.27, P=0.060, see Fig. 6 B).

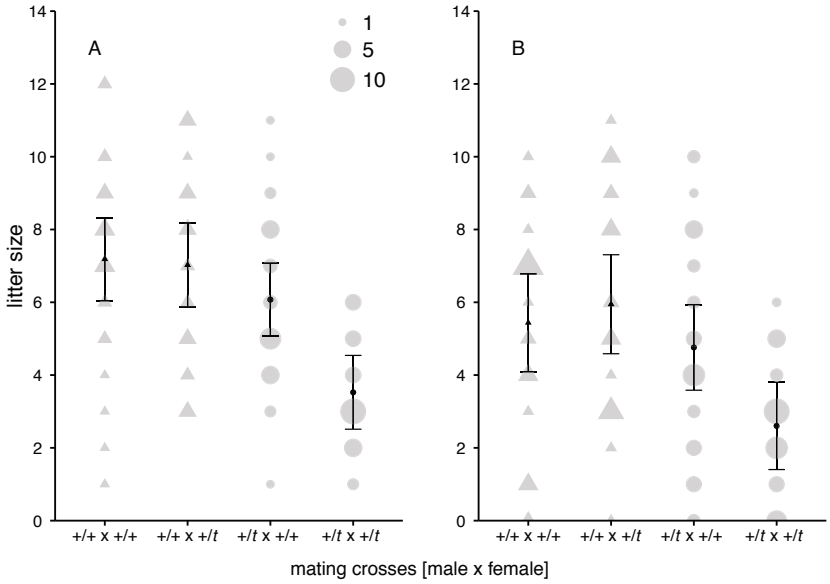


Figure 6: Litter size for all mating crosses (male genotype x female genotype) between +/*t* and +/+ mice at (A) birth and at (B) weaning. Triangles represent mating crosses from the control and circles from the experimental treatment. Model estimates and the 95% CI of the mean are displayed (LMM). Raw data are plotted in grey and the point/triangle size indicates the sample size for a certain litter size (N=104 litters, excluding 8 litters from 4 communal nests that were born on the same day and whose litter size at birth was unknown).

*Propensity to Cooperate*

A total of 112 litters were born in 25 social groups (14 groups in the experimental treatment and 11 groups in the control treatment). 94 of the litters were raised in 47 communal nests. The remaining 18 litters were solitarily reared, meaning that no other litter was born within 17 days before or after its birth (10 of the solitary litters were raised by +/+ females and 8 by +/*t* females). The two litters in communal nests were on average 3.1 ±0.6 days in age apart in the control and 3.4 ±0.7 days in age apart in the experimental treatment (mean±SE) (in 7/47 communal nests the two litters were born on the same day). At least one pup from 100 litters survived until weaning (day 23); 12 litters were lost entirely (4 solitary litters and 8 litters from communal nests).

We define a communal nest as successfully reared if at least one pup from each litter survived until weaning. Female pairs did not differ significantly between control and experimental treatment in their probability of successfully raising a communal nest (GLM: $\chi^2_{1,22}$= 20.71, P= 0.727). However, the number of communal nests (successful and unsuccessful ones) in relation to the total number of reproductive events (communal nests plus solitary litters) was significantly higher in the control than in the experimental treatment (GLM: $\chi^2_{1,23}$= 32.06, P= 0.028). Females in the experimental treatment (unequal litter size at birth) showed a reduced propensity to give birth within 17 days from each other, i.e. to form communal nests (Fig. 7).

*Infanticide*

From the 137 pups that did not reach weaning age, 131 disappeared or were found dead within their first five days. Pups found dead had wounds typically caused by adult conspecifics (bites on their head, bites in the neck region or missing body parts), as described in other studies reporting infanticide in house mice (Huck et al., 1982; Labov et al., 1985; Auclair et al., 2014b).

Pup survival did not differ between the control and the experimental treatment (the factor treatment was not retained during model selection). Moreover, larger litters in the experimental treatment (litters of +/+ females) did not have a lower survival probability and this was true whether they were the first or second born litter in a communal nest (the two-way interaction genotype:treatment, and the three-way interaction genotype:treatment:order were not retained during model selection, see Table 4).

The birth order in a communal nest however had a significant effect on the proportion of pups that reached weaning age (parametric bootstrapping, $\chi^2$=19.29, P<0.010, Fig. 8). First born litters suffered more often from a partial litter loss, and only first born litters were lost completely. In two cases, litters were born on the same day and we were not able to determine whether the pups found dead were from the first or second born litter. Of the 79 pups that died from first born litters, 65 were found dead before the second litter was born.

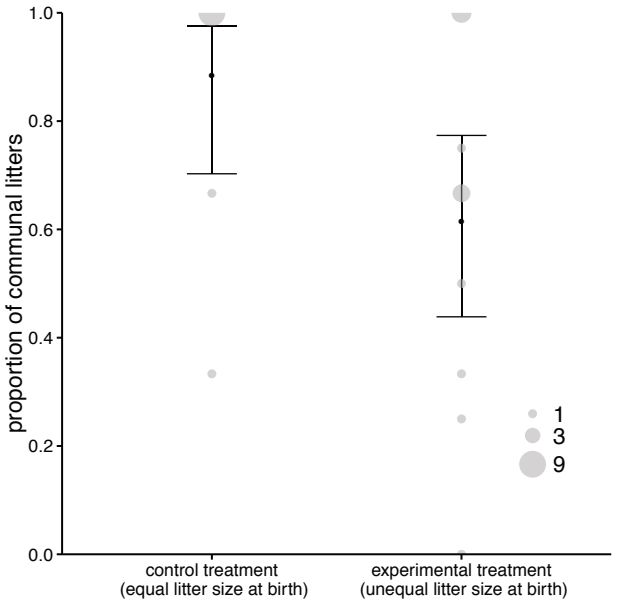


Figure 7: The proportion of communal nests (two litters born within 17 days of each other) in relation to all litters (reared communally and solitarily) raised by females in the control (similar litter size at birth) and experimental (unequal litter size at birth) treatment. Displayed are backtransformed model means and the 95% CI. Raw data are plotted in grey and the point size reflects the frequency (N=25 social groups).

If females used infanticide to equalise litter sizes, we would expect the number of killed pups in the first litter to correlate with the actual difference in litter size. Significantly more pups died before weaning in larger litters (GLMM: slope 0.27 [0.082-0.502]), but the actual differences in litter size at birth between females had no significant effect on the number of pups killed (factor was not retained during model selection).

We were unable to predict birth order among pairs of females. Neither female body weight (at the start of the experiment or after they had given birth), a female's genotype, the male's genotype (treatment) or the difference in litter size [focal litter - other litter] had an influence on a female's probability to give birth first or second. None of the included factors improved the model significantly and only the intercept was retained during model selection (N= 68 birth events as part of 36 different communal nests by 42 different females).

## DISCUSSION

Female house mice conditionally adjusted their propensity to cooperate to the potential for conflict. In a situation in which high conflict between females was expected due to pronounced differences in litter size, females raised a higher number of litters solitarily and did not cooperate. Contrary to our predictions, females did not raise two solitary litters concurrently, but instead they avoided communal

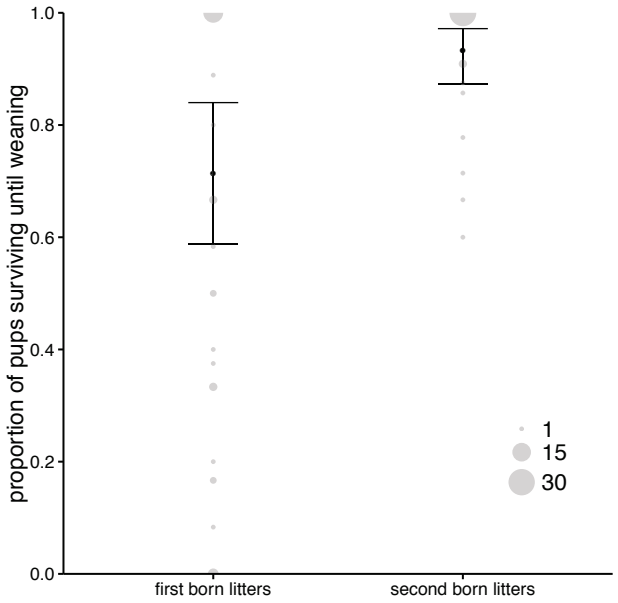


Figure 8: The proportion of pups alive at weaning for first and second born litters within communal nests. Plotted are back transformed model estimates [mean] and 95% CI of the mean. Raw data are plotted in grey and the point size indicates the frequency ($N$=80 litters from 44 different females in 40 different communal nests, excluding 7 communal nests with pups born on the same day and consequently no information available on the birthing order).

litters by not giving birth within 17 days of each other, the duration of full pup dependence. Our experiment further revealed competition over reproduction among cooperatively nursing full sisters in the form of infanticide. Female infanticide, however, did not equalise litter sizes within groups, and thus did not eliminate the conflict among the two females. The number of pups found dead shortly after birth was independent of the differences in litter size of the partners involved. Females that gave birth first in a communal nest suffered from elevated pup mortality, most probably caused by infanticide committed by the still pregnant partner.

*Propensity to Cooperate*

In the experimental treatment, under the enhanced conflict, more litters were born and raised solitarily. This was not because females avoided communal nests by giving birth in different areas of the cage system, but because only one of the females produced a litter. Such a situation arises if one female does not mate, fails to conceive or aborts her litter during pregnancy. Mating failure can either be caused by unwillingness to mate on the female's side, or it could be a consequence of the male (males were together with the females for the whole duration of the experiment). We cannot exclude that the male's genotype in the experimental treatment interfered with the females' behaviour. Females carrying the selfish genetic element ($+/t$) suffer from a reduction in litter size when they mate with $+/t$ males

and may therefore avoid such costly matings (Lindholm et al., 2013). There is evidence from other populations that $+/t$ females are able to recognise the presence of the $t$ haplotype and prefer +/+ males based on odour alone (Lenington et al., 1992). However, Lindholm et al. (2013) found, using mice from the same population as used here, that neither $+/t$ nor +/+ females showed a reduced propensity to give birth when mated monogamously with a $+/t$ male. Furthermore, Sutter and Lindholm (2015) showed that in a polyandrous situation, females presented with a $+/t$ and +/+ male readily mated with both, making it unlikely that females avoided the $+/t$ male in our experiment.

Alternatively, increased competition among females influenced the rate of successful pregnancies and consequently led to the higher number of solitary litters as observed in the experimental treatment. This would require that females were able to not only estimate their own, but their social partner's litter size prenatally and that this subsequently influenced a female's likelihood to continue the pregnancy. Avoiding communal nursing when females differ in litter size could prevent the collapse of the public good insofar as it prevents exploitation during cooperation.

Female competition could lead to only one female giving birth in two ways. First, females may abstain from reproduction if it is too costly under the given circumstances by either not implanting their embryos or through an early abortion. Secondly, it might be that females suppress their partner's reproduction for example by using aggression to instigate stress induced abortions (see review (Wasser and Barash, 1983)). We never observed two concurrent solitary litters raised in separate nests. A study using the same cage system found a high occurrence of communal nursing, but similarly never solitary litters if another female was also breeding (there were never two nests at the same time) (Weidt et al., 2007). Similar to the results here, Weidt et al. (2007) observed a number of cases where only one female of a pair gave birth, despite both having constant access to a male. We therefore assume that in our laboratory setting females did not have the option to simultaneously raise two litters solitarily within the space available; they either had the option to pool litters in a communal nest or abstain from reproduction. Withholding reproduction thus might have been the only way to avoid communal nursing.

Not to reproduce, on the other hand, might be associated with even higher costs than being the dam of the smaller litter in a communal nest. This could explain why communal nests still occurred in the high conflict treatment. Under natural conditions, females might instead of abstaining from reproduction choose to raise their litters solitarily in a separate nest, as has been described in a recent study where females where shown to raise their litters solitarily even if they had the option for communal nursing (Weidt et al., 2014). Additionally, females might be presented with not only one potential partner for cooperation, but instead have the ability to choose among several females. Indeed, there is evidence that social partner choice plays a role in a wild population of house mice. With an increasing number of available partners for cooperation, the proportion of females rearing their litters communally increased, independent of population density (Weidt et al., 2014).

An alternative explanation for communal nursing in a high conflict situation could be that we used full sisters in our experiment. Mathot and Giraldeau (2010) showed theoretically and empirically that individuals are more likely to tolerate exploitation through relatives, due to the smaller costs of exploitation (indirect fitness benefits). If females giving birth first can prevent females from joining them, we would expect higher levels of cooperation among sisters even with varying litter sizes, because females should be more likely to tolerate being joined by a related vs. an unrelated partner. Wilkinson and Baker (1988) observed that communal nursing preferentially occurs among genetically similar females in a wild population.

*Infanticide as a Competitive Reproductive Strategy*

The two litters within a communal nest had different survival rates, with higher survival in the second born litters, resulting in females weaning unequal numbers of pups, not only in our experimental treatment with elevated potential for conflict, but also in the control treatment. However, we did not find that survival was influenced by litter size differences, or our experimental treatment. As a consequence, litter sizes were not equalised after the infanticide occurred; on the contrary, the differences in survival probability often created the asymmetries in the number of pups weaned.

Pups from the older litter (first born in a communal nest) had a lower survival probability than their younger nest mates. Younger pups (second born) had a significantly higher survival probability and no entire litter was lost when older pups were already present (Fig. 8). Such an effect of the sequence of birth in a joint nest on the pups' survival probability has been seen in other studies investigating related and unrelated communally nursing female pairs (König, 1994a; Palanza et al., 2005).

Both male and female house mice commit infanticide (vom Saal, 1984), but female infanticide is more likely in this scenario. Males were shown not be infanticidal towards a female's pups if they previously mated and cohabited with that female (McCarthy and Vom Saal, 1986). In contrast, pregnant female house mice commit infanticide when confronted with pups shortly before giving birth themselves (McCarthy and vom Saal, 1985). This could explain why almost no infanticide occurred after both females have given birth. It is also consistent with an inability to discriminate their own from alien offspring as seen in a cross-fostering experiment under restricted feeding, where females did not selectively kill alien young when they needed to sacrifice some pups in order to be able to raise their litters (König, 1989a). Based on these findings and in agreement with our results, killing offspring is only expected to occur before a female gives birth to her litter to avoid killing own young. Killing some of the other female's offspring seems to be a wide spread strategy in mammals and birds to competitively bias reproductive success to one's own benefit (Andersson and Eriksson, 1982; Koenig et al., 1995; Hansson et al., 1997; Young and Clutton-Brock, 2006).

*Females Benefit Unequally*

Communal nests were mostly formed sequentially (less than 15% of communal nests were composed of litters born on the same day); one female gave birth first and as a consequence risked losing part of her litter before the other female joined. No relationship between the relative litter size and the probability to give birth first was found. Dominance among the females might determine the order in which they contribute to a communal nest and consequently which of the females is going to benefit more. Laboratory studies, however, did not reveal any signs for behavioural dominance among pairs of cooperating full sisters (König, 1994a). As soon as the litters in a communal nest are mixed, females invest according to the total number of pups in the joint nest (Ferrari et al., 2015) and have only limited options to prevent exploitation by the social partner. Aggression of highly pregnant females towards their partner's pups thus seems to be the most important mechanism to prevent exploitation and to gain reproductive benefits. However, only the female giving birth second in a communal nest can follow such a strategy. A study over a longer time period would help to determine whether communally nursing females will alternate in birth order and thus gain balanced lifetime reproductive success. Considering that house mice have a rather short life expectancy (average of 196 days, (Manser et al., 2011)) and might not necessarily cooperate again with the same social partner, the probability for reproductive skew will be high. In contrast to communally breeding birds that continue laying eggs if all of theirs had been destroyed (Koenig et al., 1995; Riehl, 2011), mammals cannot add more own young to the nest. Communally nursing females therefore do not equally benefit from their cooperation, and some may even have a disadvantage compared to solitarily nursing females.

Females might be unable to prevent another female from joining the nest. Consequently they may find themselves in a "best of a bad job" situation as soon as another female joins the nest. Because they are unable to discriminate their own from alien offspring, they either have to stay and invest into the combined litter or they have to abandon their pups, which very likely would result in even higher pup mortality. Given the rather short life expectancy of house mice, the better option might be to stay and raise the communal nest, because the costs of staying may still outweigh the costs of abandoning a litter. Communal nesting and communal nursing may additionally provide other benefits for a female, as in better protection of pups against infanticide by non-group members (Manning et al., 1995; Auclair et al., 2014b) or improved weaning weight of pups (Ferrari et al., 2015).

Our findings support the hypothesis that females avoid exploitation by conditionally adjusting their propensity to cooperate to the conflict potential in a public good situation. Furthermore, female infanticide revealed pronounced reproductive competition even among full-sisters.

## ETHICS STATEMENT

All experiments have been approved by the Veterinary Office Zurich, Switzerland (licence no.65/2011).

## AUTHORS' CONTRIBUTIONS

AKL, BK and MF contributed to the design of the study worked on the manuscript. MF performed the experiment and wrote the main text. All authors approved the text for publication.

## ACKNOWLEDGEMENT

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# CHAPTER III

# 3

# NO EVIDENCE FOR PUNISHMENT IN COMMUNALLY NURSING FEMALE HOUSE MICE (*MUS MUSCULUS DOMESTICUS*)

Manuela Ferrari und Barbara König

## ABSTRACT

Punishment is claimed as one mechanism to stabilise cooperation in humans, but its importance in social animals has been questioned recently due to both conceptual considerations and a lack of empirical evidence (only few published studies). We empirically tested whether there is evidence for punishment in communally nursing house mice (*Mus musculus domesticus*, direct descendants of "wild" animals). Communally breeding females pool their litters and raise all offspring together, indiscriminately caring for own and alien offspring. Such a situation resembles a public good and provides scope for exploitation if females vary in their relative contributions to the joint nest (offspring number). We allowed two females to communally breed and conducted removal experiments both in the presence and absence of pups. We aimed to test whether reduced investment by one of the females (induced through separation from the partner and their combined offspring for 4 and 12 hours) leads to increased aggression by the other female after the reunion. We found no evidence for punishment, on the contrary, females increased socio-positive behaviours. The costs of losing a partner in a communally breeding species might be to high and hinder the evolution of punishment. Our findings add to a growing list of examples questioning the role of punishment in cooperating non-human animals and emphasise the importance of empirical testing of its assumptions and predictions.

*PloS ONE, submitted*

## INTRODUCTION

Cooperative offspring care is a wide spread phenomenon in different taxa (Brown, 1978; König, 1997) and refers to the situation in which individuals help to raise offspring that is not their own. Reproductive skew varies among caring individuals, ranging from despotic systems with one breeding pair and several non-reproducing helpers (cooperative breeding), to egalitarian groups with several breeding females raising their offspring together in one nest (communal breeding) (Solomon and French, 1997; Koenig and Dickinson, 2004; König, 2006).

Caring for another female's offspring and especially nursing non-offspring requires an evolutionary explanation, considering the high energetic costs of parental care in general and in particular of lactation in mammals (Clutton-Brock et al.,

1989). Cooperation in these situations is expected to be evolutionary stable only when individuals gain indirect or direct fitness benefits (Hamilton, 1964a,b; West et al., 2007).

Communal breeding or nursing seems at first less ridden with conflict, because all females involved gain direct fitness benefits (in the form of reproduction), even more so in species in which females preferentially nurse their own young (Jesseau et al., 2009). However, in many species, females indiscriminately nurse all young in the nest and are unable to discriminate between own and alien offspring (Watkins and Shump, 1981; Holmes and Sherman, 1982; König, 1989a; Koford et al., 1990; Ferrari et al., 2015). Different contributions to the joint nest in terms of offspring numbers result in different benefits for the females involved. If a female contributes fewer offspring to the joint nest, she will overinvest. Her partner, on the other hand, will underinvest relative to her contribution (Ferrari et al., 2015). Adjusting parental care to the expected fitness benefits (i.e. the expected contribution to the nest) is a beneficial strategy to avoid overinvestment and can be observed in birds and fish, with males adjusting their overall investment to the rate of extra pair paternity (Burke et al., 1989; Chuang-Dobbs et al., 2001; Rios-Cardenas and Webster, 2005). They reduce feeding behaviour when the proportion of their own offspring in the nest is smaller. In a similar way, in species that cannot discriminate own from alien offspring, females could reduce their overall investment when their contribution to the joint brood or litter is smaller than their partner's. Under certain conditions females may even benefit by abandoning a litter and leave all the maternal care to the other female (intraspecific brood parasitism).

Still, a female might be unable to leave her social group since the territory provides access to safe shelters and food, and is defended against non-group members. Punishment or aggression by other females might enforce their partner's continued cooperation analogous to the cooperative breeding "pay-to-stay" hypothesis. The pay-to-stay hypothesis assumes that subordinate helpers in a cooperatively breeding group have to pay rent in the currency of investment into the offspring to be allowed on the territory, or as a member of the group (Gaston, 1978; Balshine-Earn et al., 1998). In birds (Mulder and Langmore, 1993), fish (Balshine-Earn et al., 1998) and invertebrates (Reeve, 1992), it has been shown that the dominant pair (or the dominant male alone) punishes "lazy"helpers, therefore stabilising cooperation. Raihani *et al.*(Raihani et al., 2012) challenged those findings because of lacking evidence that punishment changes the behaviour of the target individual, which is necessary for such a mechanism to work. However, Fischer *et al.* (Fischer et al., 2014) argue in their recent paper that they found evidence in a cichlid species (*Neolamprologus pulcher*) that punishment indeed increased helping behaviour in small groups, where the dominant pair can monitor individual helping behaviour of subordinates. More recently, Leighton and Meiden (Leighton and Meiden, 2016) claimed that sociable weavers (*Philetairus socius*) increased cooperative behaviours after suffering aggression.

In the communal breeding or nursing scenario, females might equally only be tolerated in a group if they provide maternal care, therefore forcing them to cooperate. The situations is different from the classical pay-to-stay scenario in several

ways, foremost the female's continued presence in the group does not inflict costs on the other females, not unless the female reduces her investment. We therefore do not aim to test the hypothesis directly, rather to draw parallels and test whether punishment might also serve to stabilise cooperation among communally breeding females, by preventing females to do less than their fair share of investment. House mice are a good study species to test whether there is evidence for coercion in a communal breeder, analogous to the examples above from cooperatively breeding species. Female house mice show two breeding strategies, rearing their young either solitarily or together with one or several other females in a communal nest (Sayler and Salmon, 1971; Manning et al., 1995; König, 1997; Auclair et al., 2014a). Females invest milk according to the total number of offspring in the nest and not their own litter size (Ferrari et al., 2015), resulting in females benefiting unequally if they differ in litter size. Females seem unable to discriminate between own and alien young, making it impossible for them to selectively nurse only own young (König, 1989a).

Given the controversial discussion of punishment as a stabilising factor for cooperation in animal systems, we empirically tested a situation of conflict in wild house mice. Since conflict is expected to be highest among unrelated individuals, we used previously unfamiliar and unrelated females in a laboratory experiment and allowed them to form a communal nest. One female was then removed from the communal nest for 4 and for 12 hours, once while females were rearing young and once as a control while they had no offspring. After 4 hours without suckling stimulus by pups milk production decreases (König et al., 1988). Nevertheless since in a wild population females may not visit their litters for even longer periods of time (communally reared pups in a wild population were found on average to be left alone 11.7 hours per day (Auclair et al., 2014a)), we also chose a second separation period of 12 hours to increase the conflict potential.

This experiment allowed us i) to asses how females reacted after the "lazy" partner returned and ii) to quantify whether the remaining female compensated for the absence of her partner by increasing the amount of time she spent nursing. If punishment or coercion play a role in stabilising cooperation among communally nursing females, we expected increased aggression after the removed female is returned only in the presence, but not the absence of pups.

## MATERIAL AND METHODS

### *Animals and Husbandry*

We used F1 to F3 descendants of wild caught house mice as study animals. The population of origin lived near Zurich (Switzerland), see (König and Lindholm, 2012) for more information. Mice were kept in the laboratory under a standardised light:dark cycle (14:10h, light on at 05:30 hours CET and constant temperature of 22-24°C). Experimental mice originated from monogamously kept breeding pairs and stayed in the parental cage until weaning (day 23). Afterwards they were kept in Macrolon Type III cages (23.5x39x15cm) together with same-sex siblings

until the beginning of the experiment. Food (laboratory animal diet for mice and rats, no. 3430, Kliba) and water were provided *ad libitum*. Cages of all mice were enriched with papertowels and cardboard, serving both as cover and nest building material.

*Experimental design*

25 pairs of genetically unrelated and unfamiliar (reared apart) female house mice, not more than 12 days apart in age (mean age difference: 4.3 days), were kept together in a cage system comprised of three Macrolon Type II cages (18x24x14cm), connected via transparent plastic tubes. Females were sexually mature but naive at the beginning of the experiment, ranging in age from 44 to 117 days (mean±SE: 69.2 ±4.5 days). After two weeks an unrelated and unfamiliar male (mean±SE: 96±9.5 days) was introduced to each pair of females. On day 16 after the introduction, the male was removed again from the cage system (see Fig. 9, A for a detailed timeline). We checked the cages daily for new litters and documented the occurrence of the first communal nest. Female weight and body condition were assessed at the onset of the experiment, at the introduction of the male, and at the end of the experiment.

*Female body condition*

Females were regularly checked for signs of aggression. According to Swiss animal law, animals were separated in the case of aggression that did or might result in severe injuries, indicated by the occurrence of bleeding open wounds on the animal's back or tail. However, house mice also frequently bite each other in the tail during aggressive interactions without serious consequences. Such harmless bites result in small scars visible on the tails. We categorised the occurrence of such scars the following way: 0) No scars on the tail, 1) one or very few scars visible, 2) several scars or tip of the tail missing, and 3) many scars and/or fresh bites on back and tail. As described before, mice falling into the third category were immediately separated from their partner and the experiment stopped. The occurrence of small scars or wounds was documented at the beginning of the experiment, immediately before the introduction of the male and at the end of the experiment.

*Removal*

One of the two females within a pair was randomly assigned to the category "removed", the other female was categorised as "resident" female. During a removal the female of the "removed" category was separated by enclosing her in one of the three cages, with the help of a shutter in the tube connecting the cages. Both females therefore remained in their home cage system and still had olfactory, auditory and partially even visual contact with each other. Such procedure is expected to minimise the stress for the separated animal. The "resident" female remained in the larger part of the cage system (two cages), and the nest was always located

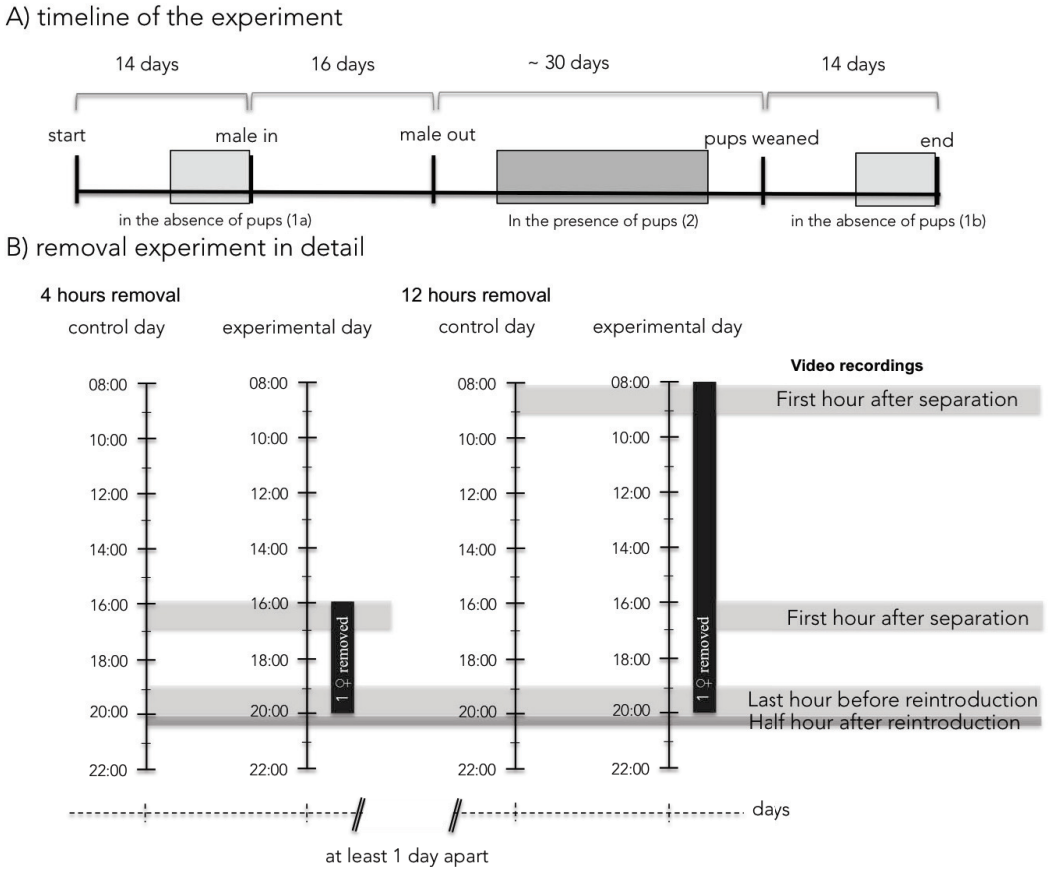


Figure 9: A) Highlighted are the periods during which the removal experiments were conducted in the absence of pups (for half of the trials in period 1a, for the other half in period 1b) and in the presence of pups (period 2) were performed. B) Detailed plan of the actual removal experiments and the corresponding video recordings used for behavioural analyses. The videos before the reintroduction of the "removed" female (first hour after separation and last hour before reintroduction) were only analysed for the "resident" females and only in the presence of pups.

within her part. Females produced nests with the provided paper towels also while they were not breeding.

Two removal experiments were conducted over the duration of the experiment; once in the absence of pups (time period 1a or 1b, see Fig. 9, A) and once in the presence of pups (time period 2, see Fig. 9, A). For half of the pairs the removal in the absence of pups took place before the introduction of the male (during the second week after the onset of the experiment) and in the other half after all offspring were weaned and removed from the cage (when 23 days old). One removal experiment comprised two separate removal events, first over 4 and then over 12 hours, with at least two days separating them. Fig. 9, B describes the removal in more detail.

*Behavioural analyses*

Videos for behavioural analyses were recorded during the removal (60min each) and after the reintroduction of the "removed" female (30min, see Fig. 9, B). Recordings were conducted on the day of the removal and during the same time periods on the day before as a control. The change in the behaviour in comparison to the control day [experimental day - control day] was used for further analysis. By analysing the difference and not the absolute values, we minimised confounding effects by other factors, such as the number of pups females had or the age of the pups during the removal.

Table 5 summarises the behaviours documented during the periods of observation in the absence and in the presence of pups.

Table 5: Behaviours recorded in the observation sessions (after reintroduction and during the removal) in the presence and absence of pups.

| behaviour | description |
|---|---|
| **After the reintroduction of the "removed" female (in the presence and absence of pups)** | |
| SOCIO-POSITIVE BEHAVIOURS | |
| time spent resting with body contact | total time two females spent resting with bodycontact [s] |
| allogrooming | occurrence of allogrooming bouts within 30min |
| SOCIO-NEGATIVE BEHAVIOURS | |
| biting | number of times a female bit the other |
| chasing | number of times a female chased the other |
| NEUTRAL BEHAVIOURS | |
| sniffing nose | number of times a female sniffed the other mouse's nose |
| sniffing anogenital region | number of times a female sniffed the other mouse's anogenital region |
| nursing (only in the presence of pups) | total time a female spent nursing the young [s] |
| **During the removal (only in the presence of pups)** | |
| nursing | total time the "resident" female spent nursing the young [s] |

*Statistical analyses*

All statistical analyses were performed with R 3.02 (R Core Team, 2015). Linear (or generalised linear) models ((G)LM) and Linear mixed models (LMM) were used for analysis. Mixed models were performed with the package lme4 (Bates et al., 2014). Fulfilment of model assumptions was tested visually and parametric bootstrapping was used to assess the significance of fixed effects. The package psych (Revelle, 2015) was used to perform a maximum likelihood factor analysis.

The behaviours recorded from both females after the reintroduction of the "removed" mouse were first analysed with a maximum likelihood factor analysis,

following the methodology described in (Budaev, 2010). In a first step, the correlation matrix between the six behavioural traits observed was tested for suitability to conduct a factor analysis. Both the Bartlett's test of sphericity ($\chi^2$=439.61, df=15, p<0.0001) and the Kaiser-Meyer-Olkin factor adequacy test (KMO=0.59) indicated the data's suitability to continue with the factor analysis.

A second, parallel analysis (Budaev, 2010) suggested to conduct the factor analysis with two factors. The matrix of loadings was rotated (varimax rotation) to obtain orthogonal factors. A detailed table with the loadings on both factors can be found in the appendix (A). The behaviours chasing and biting loaded heavily on factor 1 (0.99 each), together with a moderate loading of sniffing at another mouse's anogenital region (0.46). We consequently interpreted factor 1 as socio-negative behaviours and the scores on factor 1 were used for further analyses. In contrast, sniffing at another mouse's nose (0.71) and grooming (0.33) loaded on factor 2. We interpreted factor 2 as socio-positive behaviours and used the scores for further analyses. Sniffing at another mouse's anogenital region loaded both on factor 1 (0.46) and factor 2 (0.63), indicating for it to be a neutral behaviour in relation to the quality of the relationship between two females.

### *Ethical Note*

The experiment has been approved by the Veterinary Office Zurich, Switzerland (licence no. 90/2014).

## RESULTS

### *Compatibility of female pairs*

Twelve out of 25 pairs of females had to be separated before the first female gave birth, because of aggression between the females. Eight pairs were separated within the first five days of the experiment and four were separated after the introduction of the male. In 11 of the remaining 13 pairs both females gave birth and formed a communal nest. In one pair each, females failed to conceive, or one female died while giving birth.The first communal nest on average was formed after 63.9±8.8 days (mean±SE), with a total litter size ranging from 7 to 14 pups (mean±SE: 12±0.7 pups).

Neither the initial difference in weight nor in age between the two females had a significant effect on a pair's probability to be compatible (meaning that females did not have to be separated due to aggression) (GLM, weight difference: $F_{1,22}$=33.02, P=0.79, age difference: $F_{1,21}$=29.53, P=0.10).

### *Female body condition*

There was no significant difference between "removed" and "resident" females in the number of scars on their tails at the end of the experiment (Wilcoxon

signed-rank test, N=22, W=65, p=0.76). Overall females had more scars at the end of the experiment compared to before the introduction of the male (LM, $F_{1,40}$=9.25, p=0.004). This was, however, independent of whether a female was the "removed"or the "resident" female (LM, $F_{1,40}$=0.18, p=0.67), indicating that the removal did not result in increased aggression towards the "removed" female.

*Socio-negative and socio-positive behaviours after reintroduction of the removed female*

We found no evidence that the removal and later reintroduction of a female significantly influenced the occurrence of socio-negative behaviours in the presence of pups. Only the "resident" female in the absence of pups showed increased socio-negative behaviour that was significantly different from 0 (confidence interval of the mean did not cross 0, see Fig. 10, A). There was, however, no significant difference between the "removed" and the "resident" female ($\chi^2_1$=4.48, p=0.133) in socio-negative behaviour, nor was there a significant interaction between whether a female was "removed" or not and the presence or absence of pups (LMM, $\chi^2_1$=2.25, p=0.166).

The increase in socio-negative behaviours in "resident" females in the absence of pups might have to be taken with caution, because it is mainly driven by two outliers. When excluding those two, the confidence interval does no longer cross 0 (mean [95%CI], 0.01 [-0.11 – 0.14]). Neither the duration of the removal (LMM, $\chi^2_1$=0.1.55, p=0.250) nor the presence or absence of pups (LMM, $\chi^2_1$=4.40, p=0.141) significantly influenced aggression shown by females.

The change in socio-positive behaviour after a removal in comparison to the previous control day is illustrated in Fig. 10, B. "Resident" females increased their socio-positive behaviour towards the partner after her return significantly more than "removed" females ($\chi^2_1$=11.17, p=0.006). The duration of the removal (4 or 12 hours; $\chi^2_1$=2.30, p=0.172) and the presence or absence of pups had no significant effect on the change in behaviours in comparison to the control day (LMM, $\chi^2_1$=0.43, p=0.82).

*Maternal care*

*Effect of a separation on the time females spent nursing their young after the reintroduction*

Females that were removed in the presence of pups tended to increase the amount of time they spent nursing [s] after the reintroduction (mean [95%CI], 404s [-30.72 – 851.70]). During the same time, "resident" females significantly decreased the time they spent nursing in comparison to the control day (-406.1s [-833.75 – -10.80]. Overall, the change in the time spent nursing tended to be more positive in the 12 hours vs. the 4 hours removal (LMM, $\chi^2_1$=6.602, P=0.053), with "resident" females not significantly decreasing their nursing effort after the 12 hours removal (see Fig 11, A).

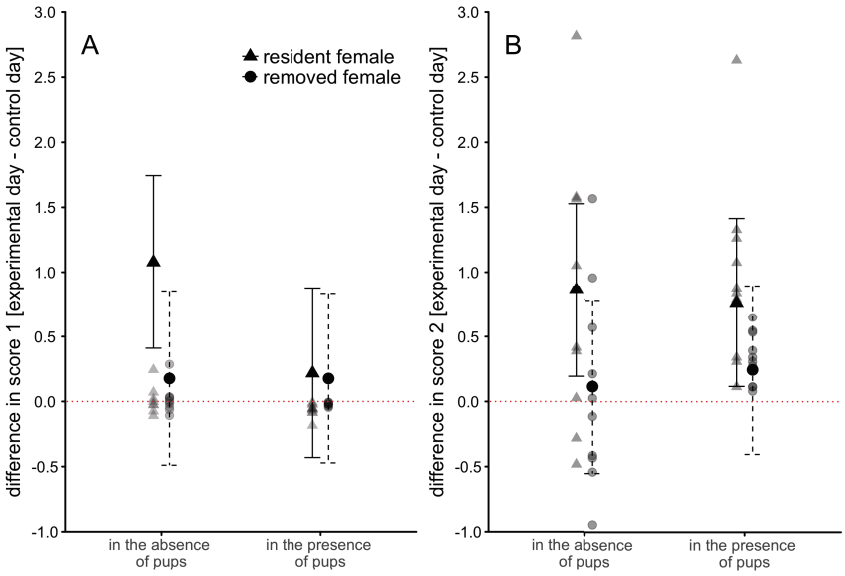


Figure 10: Change in the occurrence of A) socio-negative and B) socio-positive behaviours (factors 1 and 2 from a maximum likelihood factor analysis) shown by communally nursing female house mice after one female had been removed prior to the observation compared to control observations the day before (without a removal). Displayed are back-transformed model means and the 95% CI of the mean for the 4 hours removal only, because there was no significant difference between the 4 and 12 hours removal. Raw data are plotted in grey. Two outliers for the "resident" female in the absence of pups are omitted from the figure (they would be at 6.5 and 11.3; the values were nevertheless included in the statistical analyses).

### *Nursing effort of the resident female during removal*

"Resident" females remaining with the pups showed no significant increase in the time spent nursing in the absence of their partner in the first hour after the separation (see Fig. 11, B). In the last hour before the reintroduction of the "removed" female (see Fig. 11, B) females tended to decrease their nursing effort compared to the control day. There was, however, no significant difference between the first and the last hour of the observation session (LMM, $\chi^2_1$=4.86, P=0.160). The duration of the removal (4 or 12 hours) equally did not significantly affect the time "resident" females spent nursing (LMM, $\chi^2_1$=1.29, P=0.575).

## DISCUSSION

Our results reveal no evidence for punishment or coercion as a way of enforcing cooperation among communally nursing females. Females did not aggressively punish partners for not providing maternal care during the 4 or 12 hour removal, nor did they compensate for their partners absence by increasing nursing effort. Furthermore, we found that "resident" females showed significantly more socio-

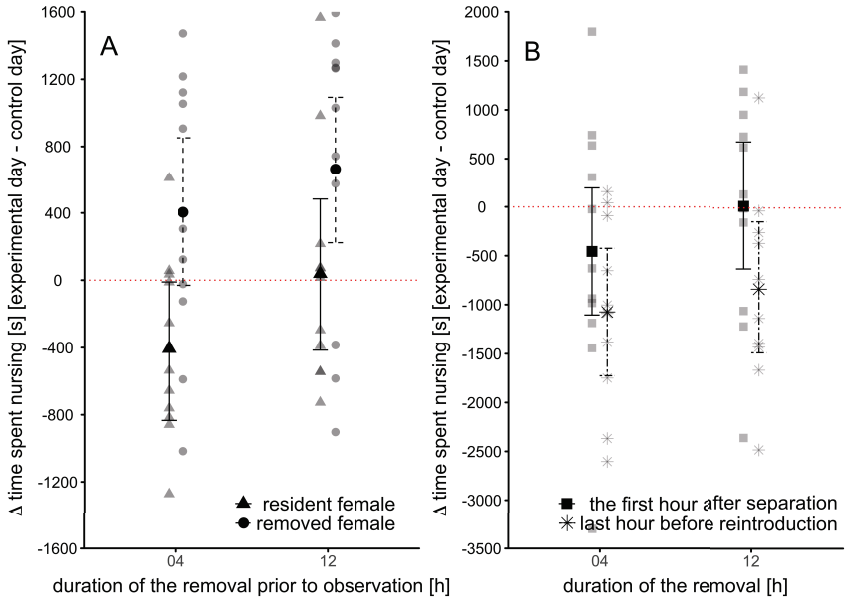


Figure 11: A) The change in time a female spent nursing in comparison to the control day after reintroduction of the "removed" female after 4 or 12 hours. B) The change in time the "resident" female (remaining with the pups) spent nursing young in the absence of her partner. One observation corresponds to the first hour after the separation, the other to the last hour before the reintroduction. Given are model estimates (means) and the 95% CI of the mean. Raw data are plotted in grey.

positive behaviours after the return of a "removed" female both in the presence and absence of pups.

*Why no punishment?*

Contrary to what we would expect if punishment served as mechanism to secure equal investment by females, "resident" females did not punish removed females after their return. Instead we found an increase of socio-positive behaviours (irrespective of whether pups were present or absent). Such a finding raises the question whether punishment is a suitable strategy in this situation. Postpunishment behaviour by the punished individual could take many different forms and only one of them (increasing cooperative behaviour) would be beneficial for the aggressor (Jones, 2002). Instead of promoting cooperation in communally nursing house mice, aggression by one female towards the other might rather result in avoidance behaviour, maybe up to the point where the harassed female abandons the nest. In that scenario, the punisher would be left to raise the relatively large communal litter on her own. House mice can increase milk production when demand is increased (Knight, 1982; König et al., 1988) but they are limited in the amount of milk they can produce and might therefore not be able to fully compensate for their partner's absence (König et al., 1988). When females are unable to sustain the whole litter, they kill some of the pups (König, 1989a), which would not be in

the interest of any of the females. Being unable to discriminate between own and alien offspring further means that females might kill own and alien offspring alike. By punishing a "lazy" partner, females would therefore risk to lose the partner, resulting in a partial litter loss and likely higher fitness costs than those associated with bearing a larger share of the maternal investment. We found an increase in socio-positive behaviours after the "removed" female was returned, both in the presence and absence of pups (see Fig. 10, B), indicating that "resident" females may encourage their partner to stay in the group, as we would expect if the costs of losing a partner are high.

We hypothesise therefore that in contrast to cooperative breeding species in which the breeding pair can raise offspring also in the absence of helpers, the costs of losing a partner in communally nursing mice are too high, which prevents the evolution of punishment.

Our findings add to a growing list of examples questioning the importance of punishment or coercion in group living animals (Jacobs and Jarvis, 1996; Raihani et al., 2012; Nomano et al., 2015; Riehl and Frederickson, 2016). Conceptually it is difficult to explain how punishment will increase helping behaviour or maternal care. Punishment will result in not performing the punished behaviour, but associating punishment with a behaviour that was not performed such as increased helping or nursing, on the other hand, is harder to conceive (Raihani et al., 2012). The punished individual could react in many different ways, but not all are necessarily beneficial for the punisher (Jones, 2002) and might therefore hinder the evolution of punishment.

There is evidence that elevated stress hormone levels increase pup feeding behaviour in a cooperative breeder (meerkat, *Suricata suricatta*, (Carlson et al., 2006)). Punishment or aggression could therefore promote cooperation by generally increasing stress levels. However, high levels of escalating aggression in the same species (meerkat) were shown to lead to evictions and stress induced abortions, rather indicating that aggression serves as a mechanism to suppress reproduction and not to promote helping behaviour (Young et al., 2006). To our knowledge there is no evidence yet linking an increase in stress induced helping behaviour to punishment.

Overall, there are only few empirical examples of punishment as a mechanism to stabilise cooperation in cooperatively breeding species (Reyer, 1986; Mulder and Langmore, 1993; Reeve, 1992; Fischer et al., 2014) and it remains still largely unknown what favoured the evolution of punishment in those species, compared to a growing list of species where punishment seems not to play a role (McDonald et al., 2008; Nomano et al., 2015; Kramer et al., 2016). Further studies are needed to determine under what conditions the evolution of punishment is facilitated, ideally also looking at the fitness consequences of punishment.

*Would females benefit from reduced investment?*

Alternatively, we could turn the question around and ask what benefits might females gain from lowering investment into a communal nest in the first place that

would make punishment necessary. We know from earlier experiments that female house mice invest (milk produced) according to the total number of pups in the nest (Ferrari et al., 2015), which provides scope for exploitation. Lowering overall investment into the communal nest when the proportion of own offspring is small would seem beneficial, but was not observed (Ferrari et al., 2015). In this experiment females that were forced to lower their investment through separation from the offspring ("removed" females), were not punished by the "resident" females" after their return. If not punishment, what may prevent a female from lowering her investment in such a situation, or in the most extreme case, to abandon the communal nest and leave the litter solely in her partner's care (intraspecific brood parasitism)? One possible explanation is that physiological constraints prevent females from lowering investment. Milk production in females is determined by the total number of pups suckling (Knight, 1982; König et al., 1988; Ferrari et al., 2015) and females might therefore have only limited options to modify their lactational effort.

Alternatively, females might not benefit from lowering their investment, or abandoning the communal nest. Reducing investment is only beneficial if the social partner compensates for the loss in investment, otherwise the overall reduction in maternal care may jeopardise offspring growth or survival and create costs that are higher than what the female gains by lowering her investment. Our data show no evidence for immediate compensation in investment by the other female (see Fig. 11, B), measured as time a female spent nursing. At the end of the removal "resident" females spent significantly less time nursing the young, which could be simply because they emptied their mammary glands.

The physiological processes involved in up-regulating milk production might take some time and would likely not be visible within the duration of even 12 hours. This might also explain why the "removed" female increased the time she spent nursing after having been returned to the offspring (see Fig. 11, A); her separation likely resulted in a build-up of milk in her mammary glands. We cannot exclude that in the longer term females would at least partly compensate for their partner's absence, as for example in case of the partner's death. Mice have a postpartum oestrus and in good condition are known to be concurrently pregnant and lactating (Norris and Adams, 1981; Krackow, 1989). Under non-food limited conditions, a female might therefore gain only little time until the birth of the next litter by abandoning her offspring. While she can avoid or lower the burden of lactation, resulting in a shorter inter-birth interval or in a larger next litter, this might not outweigh the cost of losing part of her current litter, especially if the probability to reproduce again is low.

Furthermore, females preferentially communally nurse with relatives (Wilkinson and Baker, 1988), which decreases the fitness costs of overinvesting into the joint litter, because females may gain indirect fitness benefits.

*Aggression before the onset of reproduction*

While we did not find evidence for punishment, aggression between female house mice was very pronounced before the formation of communal litters. In our study, females were unfamiliar and unrelated at the beginning of the experiment, which resulted in frequent aggression before the onset of reproduction. Aggression reflects competition over reproduction in social groups of females, which is expected to be elevated among unrelated and unfamiliar females (König, 1994b). Juvenile familiarity developed among sisters reared together rarely if ever results in such aggressive competition over reproduction (König, 1994b). Immigration of previously unfamiliar females in a group nevertheless occurs in house mice (Lidicker, 1976), which is why we chose unfamiliar and unrelated females, to maximise the conflict potential and therefore the likelihood for punishment. Almost every second pair of females (12 out of 25) had to be separated prematurely to prevent serious injuries, which likely would have resulted in the death of one of the partners. Under natural conditions, we expect one of the females to leave the group in such situations.

Females thus seemed to be very discriminative about what females they tolerated as a member of their group. Such high levels of selectiveness before the onset of breeding could indicate that females have limited options to avoid exploitation after the formation of a communal nest. Females are unable to recognise their own offspring (König, 1989a), which prevents them from preferentially nursing own pups or from removing them from the communal nest. As a consequence, once they are part of a communal nest, females can only stay and raise the joint litter or abandon their own pups, which might make partner choice beforehand very important. And indeed, there is evidence from both laboratory studies (Weidt et al., 2007) and a wild population (Weidt et al., 2014) that choice plays an important role in communal nursing. The mechanisms used in selecting a partner, however, are still unknown (Harrison et al., 2016).

## CONCLUSION

To conclude, house mice i) did not punish "lazy" partners and ii) did not increase the time spent nursing to compensate their partner's absence, at least not within a time period of 4 or even 12 hours. Punishment thus seems not to serve as a mechanism to stabilise cooperation in this communally breeding species, further questioning the overall importance of punishment in cooperative non-human animal systems (Raihani et al., 2012; Riehl and Frederickson, 2016).

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# CHAPTER IV

# 4

# FITNESS CONSEQUENCES OF FEMALE ALTERNATIVE REPRODUCTIVE TACTICS IN HOUSE MICE (*MUS MUSCULUS DOMESTICUS*)

Manuela Ferrari, Anna K. Lindholm und Barbara König

## ABSTRACT

Alternative reproductive tactics are defined as discrete differences in morphological, physiological and/or behavioural traits associated with reproduction, which occur within the same sex and species. Female house mice provide an example for alternative reproductive tactics in females. They show two different breeding tactics; females can either rear their young solitarily, or together with two or even more females as part of a communal litter. Communally nursing females do not distinguish between own and alien offspring and care for all pups indiscriminately. We assed the fitness consequences of communal and solitary nursing in a wild population, trying to understand how the two tactics can be evolutionarily stable. Females switched between the two tactics (with more than 50% of all females having two or more litters using both tactics), indicating that communal nursing is a plastic reproductive tactic and not fixed over a female's lifetime. Communal nursing resulted in reduced pup survival and therefore negatively impacted female reproductive success, with the females rearing a higher proportion of litters solitarily being more successful. Older and likely heavier females were more likely to rear their litters solitarily, indicating that communal nursing is a condition dependent alternative reproductive tactic. Younger females might not be able to rear their litter solitary and therefore opt for communal nursing as a "best-of-a-bad-job" strategy, even at the cost of increased pup mortality.

*to be submitted*

## INTRODUCTION

Reproductive success is a major component of an individual's fitness. Understanding what causes its variation and how an individual may maximise its lifetime reproductive success is therefore crucial. In many, especially group living species a large reproductive skew can be observed, with few individuals monopolising reproduction (Hager and Jones, 2009). High reproductive competition and variance in reproductive success in some instances led to the evolution of alternative life-history trajectories within the same population, with individuals using an alternative way to optimise their reproductive success (Brockmann et al., 1979).

Alternative reproductive tactics (hereafter ARTs) are defined as discrete differences in morphological, physiological and/or behavioural traits associated with

reproduction, which occur within individuals of the same sex and species (Gross, 1996; Taborsky et al., 2008). High reproductive competition and situations in which individuals can exploit the investment of same-sex competitors are expected to favour the evolution of ARTs, or more generally, situations in which individuals can improve fitness by using a different reproductive strategy. ARTs were hypothesised to have a higher prevalence in males, because high variation in fitness within the same sex is more commonly found in males and furthermore females usually cannot avoid high investment altogether (egg production, gestation etc). In agreement with this prediction is the large number of ARTs described for males in many different species (Shuster, 2008; Brockmann, 2008).

More recently, however, increasing focus on female competition revealed evidence for its prevalence and importance (Clutton-Brock, 2009; Stockley and Bro-Jørgensen, 2011), suggesting that ARTs might be more common among females than previously assumed (Taborsky et al., 2008). Typical ARTs described for females include conspecific brood parasitism (Yom-Tov, 1980; Yanagisawa, 1985; Field, 1992; Zink, 2003), the exploitation of nests built by female conspecifics (Brockmann et al., 1979; Field, 1994) or joint breeding (Scott and Williams, 1993; Schradin et al., 2012).

House mice provide an example for alternative reproductive tactics in females. They show two different breeding tactics; females can either rear their young solitarily, or two or even more females pool their litters in one nest and raise the offspring together (Sayler and Salmon, 1969; Wilkinson and Baker, 1988; König, 2006). Females in those situations nurse all pups indiscriminately (Ferrari et al., 2015) and are apparently unable to distinguish between own and alien offspring (König, 1989a). Communal nursing was shown to increase female lifetime reproductive success in the laboratory, with communally nursing sisters weaning a higher number of pups over an experimental lifespan of 6 months than solitarily nursing females (König, 1994a).

There is only little information available about the fitness consequences of communal nursing in free living populations. Manning et al. (1995) found evidence in a semi-natural population for increased pup survival in communal litters and similarly, Auclair et al. (2014b) found that communal nursing indirectly increased pups survival through polyandry driven benefits against male infanticide. Even though communal nursing has been associated with benefits in the laboratory and to a lesser degree in the wild, solitary nursing can still be observed in wild populations. Females were found to rear their offspring solitarily although other females in the same group had depended offspring at the same time (Weidt et al., 2014), indicating that females might not always benefit from pooling their litters. Communal nursing further was shown to provide scope for exploitation in the laboratory (Ferrari et al., 2015) and does not necessarily always benefit all the females involved, which makes it even more important to quantify its consequences in a wild population.

Understanding the evolution and or maintenance of an alternative tactic requires knowing the fitness consequences associated with the different tactics. There are only few empirical studies in which the fitness of individuals using different

alternative tactics was analysed in the wild (Lank et al., 1995; Brown and Brown, 1998; Schradin and Lindholm, 2011), even less so in females (Brown and Brown, 1998). Alternative tactics that are genetically determined can only be evolutionarily stable when they on average result in similar fitness, or are negatively frequency dependent (Shuster and Wade, 1991). There are, however, circumstances under which plastic alternative tactics can be evolutionarily stable even if they vary in terms of fitness. This can occur if tactics are condition dependent Gross (1996), as in a Mediterranean wrasse species (*Symphodus ocellatus*; (Alonzo et al., 2000)).

In many contexts and species the underlaying mechanisms causing alternative reproductive tactics are unknown and Taborsky et al. (2008) argue therefore that it might be more useful to use the term alternative reproductive tactic unless the underlaying mechanism are well enough studied. ARTs can be genetically determined and are then sometimes referred to as alternative strategies (Schradin et al., 2012). As a contrast there are species with a single strategy (i.e. decision rule) resulting in plastic alternative tactics, that can be fixed over an individual's lifetime, occur simultaneously or sequentially after each other (Schradin et al., 2012).

Analysing the fitness consequences of alternative reproductive tactics and whether they are plastic or fixed over an individuals lifetime can help to shed light on the underlying mechanisms, and more importantly, help explain how an alternative tactic is maintained in the population. We aimed to i) quantify the occurrence of two female alternative reproductive tactics in a wild population of house mice and ii) to calculate the associated fitness consequences. Using data from a long term study allowed us to follow individual females over their lifetime and document what tactic they used and also to analyse its effect on their reproductive success, consequently helping us to shed light on the conditions that favour the evolution and maintenance of female ARTs.

## MATERIAL AND METHODS

### *Study population*

We used data collected as part of a long term project on wild house mice, located in a barn close to Zurich, Switzerland. A free-living mouse population was set up in a barn in 2002 and has been intensively studied ever since (for a detailed description see (König and Lindholm, 2012). The barn is closed against larger predators, but allows mice (or other small animals) to enter and leave freely. Wooden and plastic barriers structure the 72m$^2$ barn into four major sections, that nevertheless can be crossed by the mice through holes. Food, water and nest building material (straw and hay) are provided at libitum at several places throughout the barn. 40 artificial nest boxes (10 per sector) serve as shelters and breeding sites for the mice. The population set-up is supposed to resemble the natural habitat of commensally living house mice in middle Europe, which is why food is provided by humans, even though it is a free-living population.

All adult and subadult mice are regularly captured (every 6 to 8 weeks) and their sex, weight and reproductive status (for females whether they are pregnant or lac-

tating) was documented. During such population monitoring, adults of minimally 18g were injected with a transponder (RFID tag; Trovan ID-100A implantable microtransponder: 0.1 g weight, 11.5 mm length, 2.1 mm diameter) for individual identification, and a tissue sample (ear punch) was collected for genotyping and pedigree analyses. All mice found dead in and around the study site were recorded and tissue samples were taken for genetic analyses. Capturing all mice in regular time intervals allowed us to estimate the population size. Tagging of adults further allowed regular documentation of their presence in the barn, either in nest boxes or in shelters (with the help of hand-held readers or by an automatic antenna reading device attached to each entrance to the nest boxes; for details see König et al. (2015)). We thus were able to determine for those adults, that were not found dead, the time they left the barn (date last seen).

*Monitoring reproduction*

The 40 nest boxes and all shelters were checked at least every 13 days for the occurrence of new litters. All litters born and documented between January 1, 2007 and December 31, 2011 were used for this study. Pups found were aged (based on morphological traits, see König and Lindholm (2012)), weighed and each litter was given a unique code (LitterID). Furthermore, we recorded whether the litter was found alone in the nest box (solitary) or communally with one or several other litters in the same nest box. A communal litter was defined as a nest (nest box or shelter) in which pups of different ages were combined (morphological differences allowed us to tell pups apart when they differed in age by 1-2 days) and thus had more than one mother. Litters sharing the same nest box or shelter were always part of a communal litter; the confined space in a nest box did not allow for occupancy by two or more solitary litters.

House mice start eating solid food when they are 17 days old and are weaned at an age of 21-23 days. All pups of at least 17 days were therefore considered subadults and were no longer considered as part of a communal litter (such definition has been used in other studies, see König (2006); Weidt et al. (2014)). From 2008, litters found at an age of 10 days or younger were tattooed in one or several paws (coloured toe tattoo, Aramis Microtattoo Systems) to facilitate discrimination between the litters. After pups were first found, litters were revisited when pups were 13 days old to again collect data on the number of living pups, their body weight and additionally to take a tissue sample (ear punch) for parentage analyses (tissue sample can only be taken when pups are 11 days or older).

After the parentage analyses (see below), pups were assigned to a GeneticLitterID, which corresponded to pups sharing the same mother. If females gave birth to litters communally at the same time, all pups were similarly aged and had therefore been registered as a "solitary" litter and all were given the same LitterID. Genetic analyses, however, later revealed that they belonged to separate mothers (and consequently received different GeneticLitterIDs). We referred to such litters as cryptic communal litters. Litters that were found for the first time when pups were older than 10 days were immediately sampled. Ideally, pups would be sam-

pled at the onset of weaning (d17), since the body weight at that age would represent maternal investment through milk. However, because pups open their eyes at day 14 and start becoming mobile (Mikesic and Drickamer, 1992), they had to be sampled beforehand not to risk being unable to find and/or identify them. Not all litters were found in the same nest box at sampling as when first found, because females may move litters after having been disturbed (own observations). This is why we did not search for litters more often. We documented whether a litter was relocated to a different nest box or shelter between first found and sampling.

This particular population of mice produced litters during almost all months of the year, but nevertheless varies seasonally in reproductive activity. The majority of litters were produced during the warmer months (April-September) (König and Lindholm, 2012), which consequently also resulted in a fluctuating population size. Temperature in the barn was constantly documented to calculate monthly mean temperature, and data collected during population monitoring events allowed us to measure population size in a given month.

*Parentage analyses and pairwise relatedness*

Parentage analysis was conducted using markers at 25 polymorphic microsatellite loci (markers: Chr1_20, D2Mit145, D3Mit278, D4Mit227, Chr5_20, D5Mit122, D5Mit352, D6Mit139, D6Mit390, D7Mit17, D7Mit319, Chr8_3, D8Mit115, D9Mit-201, Chr10_11, D10Mit230, D11Mit150, D11Mit90, Chr12_2, D12Mit91, D13Mit-88, D14Mit44, D16Mit139, D18Mit194 and Chr19_17) (Bult et al., 2007; Teschke et al., 2008). The program CERVUS 3.0 was used to assign a mother to each pup. All females seen in the barn in the 30 days prior to the birth of a pup were included as potential mothers. Pups born at the same time and assigned to the same female were given the same GeneticLitterID to allow determining litter size at sampling.

We used the same 25 markers to calculate the Wang coefficient (Wang, 2002) of pairwise relatedness among adult females in our data set with the software Coancestry (Wang (2011), https://www.zsl.org/science/software/coancestry). It allowed us to calculate the pairwise relatedness of two females relative to the genetic similarity between all females found in the reference population. All females alive in a given year were used as reference population. The analysis was done for each year separately to avoid calculating pairwise relatedness values for females whose lifespans did not overlap.

*Statistical analyses*

We used Skew Calculator 2003 (https://www.eeb.ucla.edu/Faculty/Nonacs/pi.html) to calculate the binomial skew index (B) (Nonacs, 2000, 2003) for each communal litter (for which we knew the identity of all mothers involved) to test for inequality among females concerning the number of sampled pups they contributed. Significant negative B values indicate that females were more similar in their contribution to the communal litter than expected by chance (based on the variance in contributions among all females), revealing an egalitarian situation during in-

discriminate nursing of the combined litters. Significant positive values indicate a pronounced non-egalitarian (rather despotic) situation, where one female contributed considerably more pups to the communal litter than the partner(s). Values that do not differ significantly from zero suggest that the observed distribution in reproductive output within communal litters did not significantly differ from random.

The remaining statistical analyses were performed with R Version 3.0.2 (R Core Team, 2015). We conducted (generalised) linear [(G)LMs], or (generalised) linear mixed models [(G)LMMs]. The latter were used when the data points were not independent from each other (several litters born by the same female). (G)LMMs were performed with the package lme4 (Bates et al., 2014). Fulfilment of model assumptions were inspected visually and, if necessary, data were transformed or the appropriate link function was chosen. GLMs and GLMMs with a binomial error distribution were tested for overdispersion. The factors population size and the mean temperature in the barn were included in all analyses, to control for a potential effect of seasonality on the factors of interest. We started with the full model, containing all biological relevant parameters and used the dredge function in the MuMin package (Bartoń, 2014) to calculate all possible models containing those or fewer variables. Models were ranked according to their AICc (Akaike information criterion corrected for small sample sizes) and we used an information-theoretic approach to account for uncertainty in model selection and parameter estimation. Following Grueber et al. (2011) we obtained averaged parameter estimates (full average), by averaging across all models within 4 ΔAICc of the best model using Akaike weights (w).

### *Ethical notes*

Data collection was approved by the Veterinary Office Zurich, Switzerland (licence numbers 215/2006 and 51/2010).

## RESULTS

Within the five year study period we recorded 1279 litters in the barn. From 797 of those litters we sampled pups (alive) when they were approximately 13 days old (mean age in days ± SE, 13.0 ± 0.07), which we used as a proxy for survival until weaning. Furthermore, we sampled pups found dead from another 51 litters. In total 350 females contributed to all the litters we sampled (alive or dead). The 797 sampled litters contained 3317 living pups, with pups belonging to 963 genetically distinct litters (with unique GeneticLitterIDs). Cryptic communal litters (LitterID's that contained pups from more than one GeneticLitterID) were relatively frequent, with 20% of all litters first described as solitary (based on morphology of pups) revealed to be cryptic communal litters after genetic analyses, and 27 % of all communal litters contained more litters than we had assumed from differences in morphology among pups.

All pups from 730 of the altogether 797 litters were assigned a mother with a certainty of 95% or more. Due to a rather high number of unassigned pups in the remaining litters, when using this conservative threshold, we additionally included 67 litters that contained one or several pups that were only assigned to a mother with a confidence of at least 80%. 123 pups could not be assigned to a mother, resulting in a slight underestimation of the litter size at sampling. Furthermore, for 451 pups found dead or alive it was not possible to determine whether they were found for the first time, or belonged to a litter we had seen before, because of contradicting tattoos, age estimates or the number of pups. We therefore omitted those pups from most of the analyses, again leading to an underestimation of the number of pups sampled in the population. Those pups were, however, included when we looked at the total number of pups sampled per female (see below).

Not all litters could be used for all analyses, because we did not have complete information for all the factors of interest. Detailed sample sizes will be given for each analyses when we present the respective results.

*Alternative reproductive tactics*

During the five year study period we sampled pups (alive) from 963 genetically distinct litters (different GeneticLitterIDs) that originated from 350 different females. 662 out of the 963 litters were sampled as a communal litter, 297 were sampled solitarily and for 3 we did not have the information about their breeding condition at sampling, which corresponds to 69% of all litters being raised communally. The proportion of litters sampled communally increased slightly over the years (see Fig. 12).

Females gave birth to 1 to 9 litters, with 30% of the females having only one litter. Of all the females that reproduced more than once, 93 reared all their litters communally, 19 had only solitary litters and 134 females used both alternative reproductive tactics. We analysed, whether female age at the time she gave birth to a litter, the mean temperature in the barn or the population density during the month the litter was born influenced the probability that a litter was raised solitarily or communally. Some litters had to be excluded (96 litters) because pups from the same GeneticLitterID were spread over several nest boxes, which made it impossible to determine the social environment they were raised in. Additionally, some litters were removed from the analyses, due to incomplete information for the factors of interest, leaving us with 862 litters.

Model selection revealed that female age was the strongest predictor of the alternative reproductive tactic used by a female. With increasing age a female's likelihood to raise pups communally decreased (see Fig. 13 and Table 6). Age correlated significantly with the number of litters a female had (Pearson's correlation coefficient=0.60, $p<0.001$, N=862), we therefore could not include female experience (number of litters) into the model as well. To disentangle whether female experience or female age caused the effect that older females reared fewer litters communally, we repeated the analyses only including a female's first litter. All females that were born and may have reproduced before 2007 were excluded from

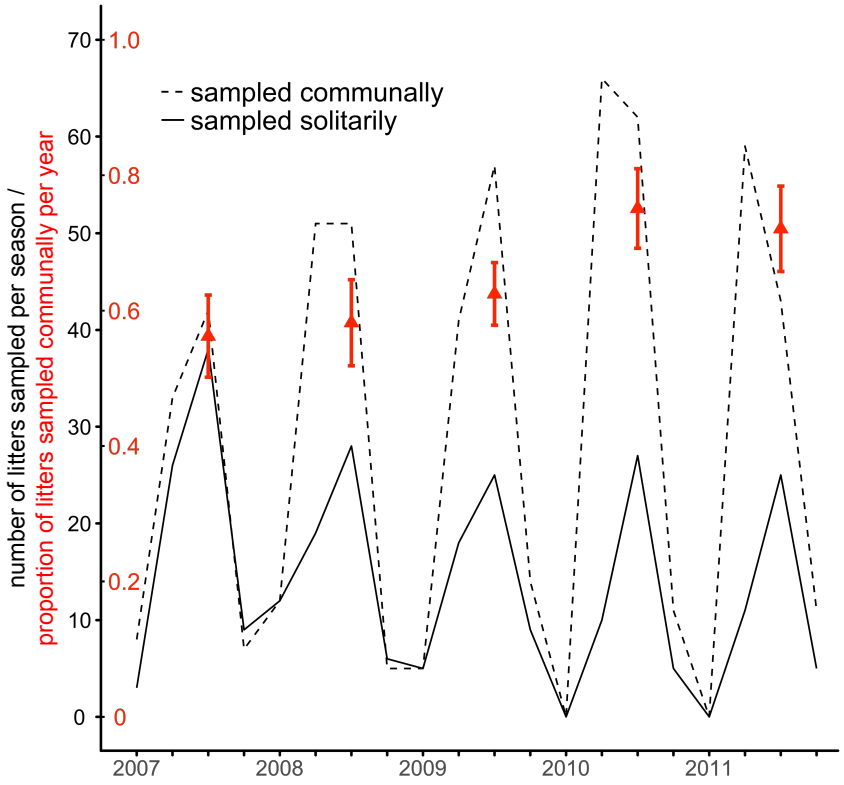


Figure 12: The number of solitary and communal litters sampled per season (spring: Mar to May, summer: Jun to Aug, autumn: Sep to Nov, winter: Dec to Feb) for all five years of the study period. Red shows the proportion of all litters raised communally for each year (mean ±SE).

the analyses, leaving us with 293 females in the data set. Even though females had all a similar level of experience because they were raising their first litter, female age still had a significant effect on a litter's likelihood of being raised communally or solitarily (averaged model estimate (slope) for female age [95%CI]: -0.74 [-1.40 – -0.12]), indicating that it was indeed age that caused the effect and not how many litters a female had already raised before.

*Female reproductive success*

We calculated reproductive success for all females born between 2007 and 2011 that reached adulthood (being tagged) and died or were last seen in the barn before the end of 2011 (N=500). 159 of the females were found dead, for the other females we do not know with certainty whether they dispersed or died outside of the barn. As a result, our measure for female lifespan is only an approximation and corresponds to the time a female spent in the population. Here we define a female's reproductive success as the number of pups weaned (sampled alive) during the time she was observed in the population. For 249 of the 500 no offspring

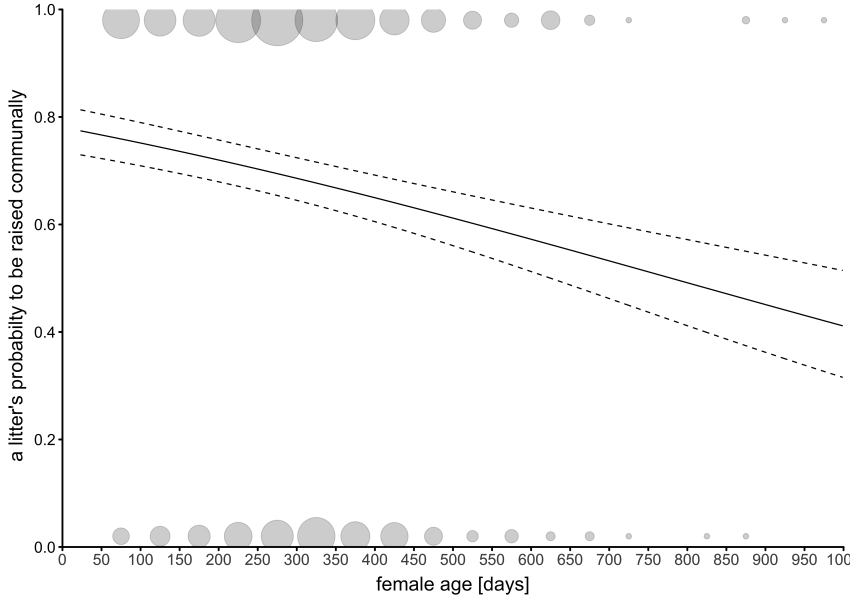


Figure 13: With increasing age, females reared more of their litters solitarily. Plotted are model estimates (mean± SE) obtained from model averaging of binomial GLMMs. Raw data are illustrated with circles and the size corresponds to the sample size (N= 862 litters).

Table 6: Model summary statistics for the factors influencing whether a litter was sampled solitarily or communally

| | (Intercept) | female age | population size | mean temp | df | AICc | ΔAICc | w |
|---|---|---|---|---|---|---|---|---|
| **Model 1** | **0.781** | **-0.456** | **–** | **–** | **4** | **1076.3** | **0.00** | **0.413** |
| **Model 2** | **0.782** | **-0.468** | **–** | **-0.178** | **5** | **1077.1** | **0.75** | **0.284** |
| **Model 3** | **0.783** | **-0.461** | **-0.169** | **–** | **5** | **1078.1** | **1.79** | **0.168** |
| **Model 4** | **0.784** | **-0.470** | **-0.099** | **-0.170** | **6** | **1079.0** | **2.70** | **0.107** |
| Model 5 | 0.786 | – | – | – | 3 | 1083.2 | 6.91 | 0.013 |
| Model 6 | 0.787 | – | – | -0.146 | 4 | 1084.4 | 8.07 | 0.007 |
| Model 7 | 0.787 | – | -0.114 | | 4 | 1085.2 | 8.83 | 0.005 |
| Model 8 | 0.787 | – | -0.055 | -0.141 | 5 | 1086.4 | 10.07 | 0.003 |
| Averaged parameters | | | | | | | | |
| Estimate* | 0.78 | -0.46 | -0.04 | -0.07 | | | | |
| Unconditional SE | 0.18 | 0.15 | 0.19 | 0.13 | | | | |
| Lower 95%CI | 0.42 | *-0.76* | -0.43 | -0.33 | | | | |
| Upper 95%CI | 1.14 | *-0.16* | 0.35 | 0.19 | | | | |
| Relative importance | | 1 | 0.28 | 0.4 | | | | |

Whether a litter was sampled communally or solitarily was used as response variable in a GLMM with a logit link function. Models within 4 AICc units of the best model are highlighted in bold and were used to calculate averaged effect sizes (*standardised on two standard deviations following Gelman (2008)). The identity of the dam of a litter (FemaleID) and the year of birth were included as random effects in all models. Factors included are defined as follows. Female age: age of female when giving birth to the litter [days]; population size: number of adults present in the barn during the month the litter was born; mean temperature: mean temperature in the barn during the month the litter was born. Abbreviations: df, degrees of freedom; w, relative model weights.

reached sample age (around d13), for the remaining 251 females we sampled between 1 and 39 pups during their lifespan in the barn (see Fig. 14).

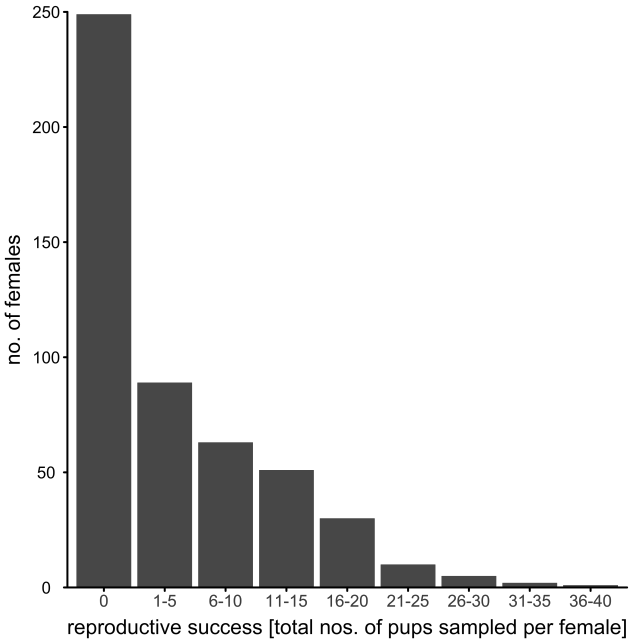


Figure 14: Reproductive success for adult female house mice in a wild population. Given are the number of pups sampled per female and their frequency in the population (N=500 females).

In a second step, we focused only on reproducing females (females that weaned at least one offspring), analysing in more details the factors that might potentially affect the variation in reproductive success observed among females. We used a linear model with the square-root of reproductive success as response variable to analyse what effect the population size and the mean temperature (during the female's month of birth), her body weight at tagging (approximation for her body condition before the onset of reproduction), her age at first reproduction, her total lifespan (i.e. the time she was observed in the barn) and how she raised her litters (proportion of litters she raised communally) had on a female's reproductive success. Our data set included 212 females that produced between 1 and 39 pups. We had to exclude the remaining females from the analyses, because we did not have information for all the factors of interest. Our measure for reproductive success only included offspring for which we knew how they were raised (communally or solitarily) and might therefore be a slight underestimation of a female's reproductive success.

Model selection revealed that lifespan in the barn had the strongest effect on female reproductive success, with longer lived females producing a higher number of offspring (see Table 7 and Fig. 15 A). Furthermore, we found that with a higher proportion of a female's litters being raised communally, she had a lower reproductive success (see Table 7 and Fig. 15 B).

Table 7: Model summary statistics for the factors influencing female reproductive success

| | (Intercept) | population size | lifespan | mean temperature | age first reproduction | strategy | weight | df | AICc | ΔAICc | w |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Model 1** | **2.895** | **–** | **1.290** | **–** | **-0.260** | **-0.337** | **–** | **5** | **582.7** | **0.00** | **0.195** |
| **Model 2** | **2.895** | **-0.167** | **1.249** | **–** | **-0.230** | **-0.316** | **–** | **6** | **583.3** | **0.56** | **0.147** |
| **Model 3** | **2.895** | **-0.206** | **1.167** | **–** | **–** | **-0.317** | **–** | **5** | **583.9** | **1.21** | **0.106** |
| **Model 4** | **2.895** | **–** | **1.205** | **–** | **–** | **-0.344** | **–** | **8** | **584.2** | **1.52** | **0.091** |
| **Model 5** | **2.895** | **–** | **1.290** | **-0.060** | **-0.238** | **-0.336** | **–** | **6** | **584.7** | **1.93** | **0.074** |
| **Model 6** | **2.895** | **–** | **1.291** | **–** | **-0.261** | **-0.336** | **-0.008** | **6** | **584.8** | **2.11** | **0.068** |
| **Model 7** | **2.895** | **–** | **1.222** | **0.137** | **–** | **-0.341** | **–** | **5** | **585.2** | **2.52** | **0.055** |
| **Model 8** | **2.895** | **-0.171** | **1.253** | **–** | **-0.233** | **-0.315** | **-0.030** | **7** | **585.4** | **2.65** | **0.052** |
| **Model 9** | **2.895** | **-0.163** | **1.250** | **-0.017** | **-0.225** | **-0.316** | **–** | **7** | **585.4** | **2.69** | **0.051** |
| **Model 10** | **2.895** | **-0.180** | **1.182** | **-0.084** | **–** | **-0.319** | **–** | **6** | **585.7** | **2.95** | **0.045** |
| **Model 11** | **2.895** | **-0.206** | **1.167** | **–** | **–** | **-0.317** | **-0.003** | **6** | **586.1** | **3.33** | **0.037** |
| **Model 12** | **2.895** | **–** | **1.202** | **–** | **–** | **-0.344** | **-0.027** | **5** | **586.3** | **3.57** | **0.033** |
| Model 13 | 2.895 | – | 1.293 | 0.062 | -0.240 | -0.336 | -0.016 | 7 | 586.8 | 4.06 | 0.026 |
| Model 14 | 2.895 | -0.209 | 1.257 | – | -0.232 | – | – | 5 | 587.1 | 4.33 | 0.022 |
| ... | | | | | | | | | | | |
| Model 64 | 2.895 | – | – | -0.028 | 0.182 | – | 0.168 | 5 | 663.1 | 80.33 | 0.000 |
| Averaged parameters | | | | | | | | | | | |
| Estimate* | 2.90 | -0.08 | 1.24 | -0.02 | -0.15 | -0.33 | -0.0 | | | | |
| Unconditional SE | 0.06 | 0.13 | 0.14 | 0.08 | 0.16 | 0.13 | 0.06 | | | | |
| Lower 95%CI | 2.77 | -0.34 | *0.96* | -0.17 | -0.47 | *-0.58* | -0.12 | | | | |
| Upper 95%CI | 3.02 | 0.17 | *1.53* | 0.13 | 0.17 | *-0.07* | 0.12 | | | | |
| Relative importance | – | 0.46 | 1 | 0.24 | 0.62 | 1 | 0.20 | | | | |

The square-root of the number of pups sampled over a female's lifetime (reproductive success) was used as response variable in a LM. Models within 4 AICc units of the best model are highlighted in bold and were used to calculate averaged effect sizes (*standardised on two standard deviations following Gelman (2008)). Factors included are defined as follows. Population size: number of adults present in the barn during the month a female was born; lifespan: time between birth of a female and her death, or until she was last seen in the barn; mean temperature: mean temperature in the barn during the month a female was born; age first reproduction: a female's age when she first reproduced; strategy: the proportion of own litters a female raised communally; weight: a female's body weight at tagging as an indicator for her condition before first reproduction.

Table 8: Model summary statistics for the effect a current litter had on the likelihood that the mother had another one

| | (Intercept) | status at sampling | population size | female age | ls at sampling | mean temp | df | AICc | ΔAICc | w |
|---|---|---|---|---|---|---|---|---|---|---|
| **Model 1** | **0.551** | **–** | **-0.611** | **-0.604** | **0.785** | **-0.665** | **6** | **979.8** | **0** | **0.732** |
| **Model 2** | **0.550** | **+** | **-0.611** | **-0.604** | **0.785** | **-0.665** | **7** | **991.9** | **2.04** | **0.264** |
| Model 3 | 0.538 | – | – | -0.650 | 0.796 | -0.814 | 5 | 992.7 | 12.81 | 0.001 |
| Model 4 | 0.543 | – | -0.658 | – | 0.759 | -0.617 | 5 | 993.4 | 13.54 | 0.001 |
| Model 5 | 0.535 | – | -0.756 | -0.557 | 0.741 | – | 5 | 993.6 | 13.71 | 0.001 |
| Model 6 | 0.515 | + | | -0.657 | 0.779 | -0.815 | 6 | 994.5 | 14.65 | 0.000 |
| Model 7 | 0.564 | + | -0.664 | – | 0.774 | -0.615 | 6 | 995.3 | 15.43 | 0.000 |
| ... | | | | | | | | | | |
| Model 32 | 0.436 | + | – | – | – | – | 3 | 11473.5 | 16.06 | 0.000 |
| Averaged parameters | | | | | | | | | | |
| Estimate* | 0.55 | 0.00 | -0.61 | -0.60 | 0.79 | -0.66 | | | | |
| Unconditional SE | 0.09 | 0.10 | 0.16 | 0.16 | 0.16 | 0.17 | | | | |
| Lower 95%CI | 0.39 | -0.18 | *-0.92* | *-0.91* | *0.47* | *-1.00* | | | | |
| Upper 95%CI | 0.71 | 0.18 | *-0.30* | *-0.30* | *1.10* | *-0.32* | | | | |
| Relative importance | | 0.27 | 1 | 1 | 1 | 1 | | | | |

Whether a female had a subsequent litter (yes or no) was used as response variable in a binomial GLMM. Models within 4 AICc units of the best model are highlighted in bold and were used to calculate averaged effect sizes (*standardised on two standard deviations following Gelman (2008)). Female identity and the year in which the first litter was born in were added as random factors. Factors included are defined as follows. Status at sampling: rearing condition at sampling (solitary or communal); population size: number of adults present in the barn during the month the current litter was born; female age: age of female at birth of her current litter [days]; ls at sampling: her current litter's size at sampling; mean temperature: mean temperature in the barn during the month her current litter was born.

### *Likelihood to have a subsequent litter and the inter-birth interval*

We analysed what effect a focal litter had on the probability that the mother gave birth to a subsequent litter (N=789 litters). Whether a female raised a litter solitar-

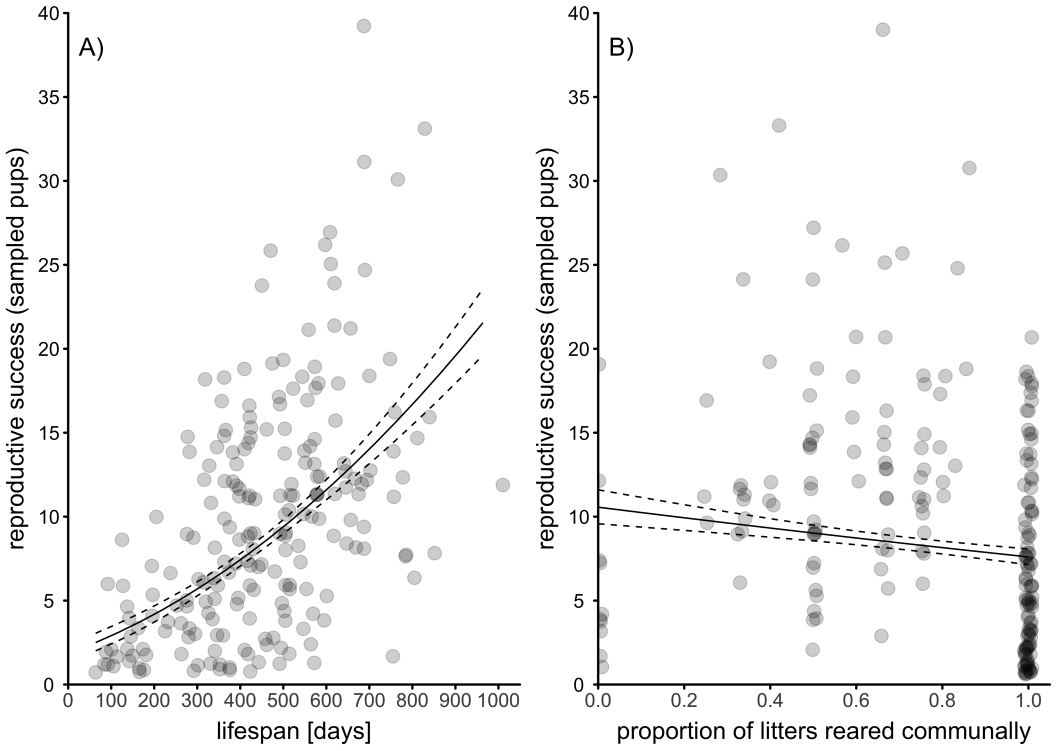


Figure 15: Female reproductive success (number of offspring sampled) in relation to A) her lifespan (i.e. the time she was observed in the population) and B) the proportion of litters a female reared communally. Plotted are model estimates (mean± SE) obtained from model averaging of LM's. Grey dots represent raw data (N= 212 females).

ily or communally had no effect on the mother's likelihood to give birth to another litter (see Table 8). The younger a female was when giving birth, and the lower the population density and mean temperature in the barn were, the more likely she had a subsequent litter (see Table 8).

When analysing the female inter-birth intervals, we found shorter intervals for younger mothers. The mean temperature in the barn, the population density and whether the last litter was sampled communally or solitarily, however, had no influence on a female's inter-birth interval (see Table 9). On average, the inter-birth interval was 66.7 ± 2.8 days [mean ± SE], ranging from 19 to 309 days (N=454 pairs of litters).

Table 9: Model summary statistics for inter-birth interval

| | (Intercept) | status at sampling | population size | female age | ls at sampling | mean temp | df | AICc | ΔAICc | w |
|---|---|---|---|---|---|---|---|---|---|---|
| **Model 1** | **3.920** | **–** | **–** | **-0.207** | **–** | **–** | **5** | **942.9** | **0** | **0.259** |
| **Model 2** | **3.920** | **–** | **–** | **-0.206** | **–** | **0.064** | **6** | **944.0** | **1.07** | **0.152** |
| **Model 3** | **3.920** | **–** | **0.045** | **-0.208** | **–** | **–** | **6** | **944.7** | **1.84** | **0.103** |
| **Model 4** | **3.920** | **–** | **–** | **-0.207** | **-0.015** | **–** | **6** | **944.9** | **2.00** | **0.095** |
| **Model 5** | **3.921** | **+** | **–** | **-0.207** | **–** | **–** | **6** | **944.9** | **2.05** | **0.093** |
| **Model 6** | **3.920** | **–** | **0.035** | **-0.207** | **–** | **0.062** | **7** | **945.9** | **3.01** | **0.058** |
| **Model 7** | **3.920** | **–** | **–** | **-0.205** | **-0.020** | **0.066** | **7** | **945.9** | **3.04** | **0.057** |
| **Model 8** | **3.923** | **–** | **–** | **-0.205** | **–** | **0.065** | **7** | **946.0** | **3.12** | **0.054** |
| **Model 9** | **3.920** | **+** | **0.045** | **-0.207** | **-0.015** | **–** | **7** | **946.7** | **3.85** | **0.038** |
| **Model 10** | **3.922** | **–** | **0.046** | **-0.207** | **–** | **–** | **7** | **946.8** | **3.90** | **0.037** |
| Model 11 | 3.920 | + | | -0.207 | -0.015 | – | 7 | 947.0 | 4.06 | 0.034 |
| Model 12 | 3.920 | + | 0.035 | -0.207 | -0.019 | 0.063 | 8 | 947.9 | 4.99 | 0.021 |
| ... | | | | | | | | | | |
| Model 32 | 3.930 | + | | 0.034 | -0.021 | 0.070 | 8 | 957.5 | 14.65 | 0.000 |
| Averaged parameters | | | | | | | | | | |
| Estimate* | 3.92 | -0.001 | 0.01 | -0.23 | -0.003 | 0.02 | | | | |
| Unconditional SE | 0.06 | 0.03 | 0.05 | 0.06 | 0.0.03 | 0.05 | | | | |
| Lower 95%CI | *3.80* | -0.06 | -0.09 | *-0.33* | -0.10 | -0.07 | | | | |
| Upper 95%CI | *4.01* | 0.06 | 0.11 | *-0.01* | 0.05 | 0.12 | | | | |
| Relative importance | | 0.19 | 0.25 | 1 | 0.20 | 0.34 | | | | |

The log of the inter-birth interval was used as response variable in a LMM. Models within 4 AICc units of the best model are highlighted in bold and were used to calculate averaged effect sizes (*standardised on two standard deviations following Gelman (2008)). The intercept represents the situation when the first litter of the pair was raised communally. Female identity and the year in which the first litter was born were added as random factors. Factors included are defined as follows. Status at sampling: rearing condition at sampling (solitary or communal); population size: number of adults present in the barn during the month the current litter was born; female age: age of female at birth of her current litter [days]; ls at sampling: her current litter's size at sampling; mean temperature: mean temperature in the barn during the month her current litter was born.

Table 10: Model summary statistics showing the effect of pairwise relatedness, the age difference between females and the age differences between litters on reproductive skew among mothers contributing to a communal nest

| | (Intercept) | age difference (females) | age difference (litters) | average pairwise relatedness | df | AICc | ΔAICc | w |
|---|---|---|---|---|---|---|---|---|
| **Model 1** | **0.001** | **–** | **–** | **–** | **2** | **-144.4** | **0.00** | **0.228** |
| **Model 2** | **0.001** | **–** | **0.030** | **–** | **3** | **-144.3** | **0.13** | **0.214** |
| **Model 3** | **0.015** | **0.0001** | **–** | **–** | **3** | **-143.9** | **0.49** | **0.179** |
| **Model 4** | **0.013** | **0.0001** | **0.026** | **–** | **4** | **-143.2** | **1.24** | **0.123** |
| **Model 5** | **0.001** | **–** | **–** | **0.002** | **3** | **-142.3** | **2.16** | **0.078** |
| **Model 6** | **0.001** | **–** | **0.032** | **0.008** | **4** | **-142.2** | **2.20** | **0.076** |
| **Model 7** | **0.015** | **0.0001** | **–** | **0.003** | **4** | **-141.7** | **2.69** | **0.060** |
| **Model 8** | **0.013** | **0.0001** | **0.027** | **0.009** | **5** | **-141.1** | **3.36** | **0.043** |
| Estimate* | 0.006 | -0.00003 | 0.013 | 0.013 | | | | |
| Unconditional SE | 0.014 | 0.0001 | 0.02 | 0.011 | | | | |
| Lower 95%CI | -0.022 | -0.0001 | -0.03 | -0.02 | | | | |
| Upper 95%CI | 0.035 | 0.0001 | 0.05 | 0.024 | | | | |
| Relative importance | – | 0.40 | 0.46 | 0.26 | | | | |

The binomial skew index was used as response variable in a LM. Models within 4 AICc units of the best model are highlighted in bold and were used to calculate averaged effect sizes (*standardised on two standard deviations following Gelman (2008)). Factors included are defined as follows. Age difference (females); absolute age difference [days] between the oldest and the youngest female contributing to the communal litter; age difference (litters): age of oldest litter minus age of youngest litter [days]; average pairwise relatedness: average pairwise relatedness between the females contributing to the communal litter [Wang coefficient (Wang, 2002)].

*Reproductive skew among communally nursing females*

We analysed 77 communal litters that contained between 2 and 4 litters, with on average 2.2 ± 0.05 females contributing to the communal litter [mean ± SE]. The binomial skew index (B) averaged over all communal litters did not differ significantly from 0 (B=0.0004, p=0.48), indicating that the observed variation in female contribution did not differ from random expectation. We nevertheless tested whether the average pairwise relatedness among females sharing a communal litter influenced the amount of skew we observed. If three or more females contributed to a communal litter, we took the average of all pairwise relatedness values. We further included the maximal age difference between the pups (age of oldest litter minus age of youngest litter), because a higher reproductive skew might be expected if pups differ in age, because killing younger pups before joining a communal nest might be easier. Additionally, the age difference between the females (age of oldest female minus age of youngest female) was included to test whether a larger age difference between females, and therefore likely also weight difference might indicate that one of the females was dominant over the other. None of the variables, however, influenced the B index (see Table 10). The age difference between the litters had the highest importance of all the variables (0.48), but still did not significantly influence skew (95%CI crossed zero). Females communally rearing their litters together were on average more closely related to each other than the average pairwise relatedness in the population (mean [95%CI]=0.28 [0.22–0.34] (Wang estimator)), but communal nursing was also observed among females that were less related than the population average (13 communal litters were formed by females that had a negative Wang estimator of pairwise relatedness, range: -0.27 – 0.78.).

*Pup survival*

We analysed pup survival in all litters that were found at least once before sampling, allowing us to calculate the proportion of pups surviving to sampling compared to the litter size when first found. If all pups of a litter died before sampling i.e. were never found again, we were unable to assign them to a mother and therefore had to exclude them from the analyses. Omitting all litters with a survival of zero resulted in an overestimation of survival rates. We included an analyses of a litter's resampling probability in the appendix as a way of estimating survival probabilities (see Appendix D). Further, all litters that were first found at sampling and all cryptic communal litters had to be excluded from the analyses, because we were unable to determine litter size when first found and consequently to calculate the proportion of pups surviving until sampling. 340 litters remained in the data set.

Litters that were smaller and older when first found had a higher survival probability (see Table 11). A litter's rearing condition further had a significant effect on pup survival rates, with the highest survival observed for solitarily reared litters (both solitary when first found and at sampling) and litters that turned solitary (communal when first found and solitary at sampling) (see Table 11). A smaller

proportion of pups survived from litters that were initially found solitary but communal at sampling, or always communal. Litters that were moved between nest boxes between two sightings also had a reduced survival probability (see Fig. 16 A and Table 11). Population density and the mean temperatures during the month in which a litter was born had no significant effect the proportion of pups surviving (95%CI of their estimates did cross zero, see Table 11).

Both solitarily reared litters and litters that turned solitary were characterised by not being joined by another litter until sampling. We therefore decided in a second step to analyse whether the number of additional new litters a focal litter was found with at sampling, influenced pup survival. With each litter added to the nest until sampling, we found a reduction in the proportion of pups surviving (see Fig. 16 B and Table 16). Whether a litter was solitary or communal when first found had no significant effect on a litter's survival rate. Similarly to the first analysis, population and mean temperatures had no strong effect on pup survival, while relocation to a different nest box and decreasing pup age reduced survival rates.

*Recruitment rate*

For all the litters that were sampled we analysed the proportion of pups that were later caught and tagged as adults in the population (N=589 litters). This does not reflect survival until adulthood, since pups may have died or left the barn as subadults, but what proportion of a litter was recruited into the population. The analyses revealed that the status at sampling (communal vs. solitary) had no influence on the proportion of a litter recruited into the population (relative low importance of 0.30). The number of recruited pups increased with decreasing mean temperature and increasing population density in the barn (see Table 12). Furthermore pups from larger litters had a higher probability to be recruited into the population (see Table 12).

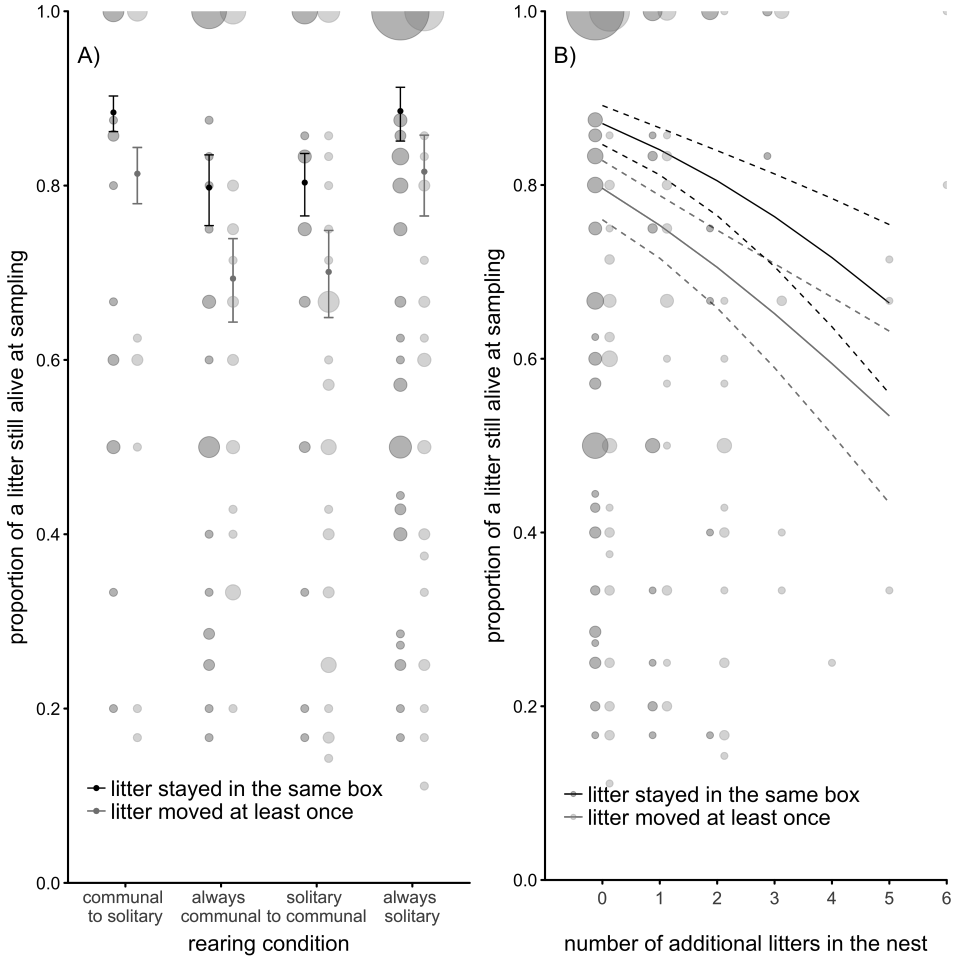


Figure 16: **A)** The proportion of pups surviving until sampling (day 13) when reared solitary or communally (the same condition both when first found and at sampling), or in litters that were solitary or communal when first found but afterwards communal respectively solitary when sampled. Dark and light grey highlights the different survival probabilities for litters that were found and sampled in the same nest box and for litters that had been moved at least once between the sightings. **B)** The proportion of pups surviving until sampling in relation to the number of additional litters in the same nest box (added after a litter was first found). Plotted are model estimates (mean± SE) obtained from model averaging of binomial GLMMs. Raw data are illustrated with circles, the size of the circles corresponds to the sample size (N= 340 litters).

Table 11: Model summary statistics for the proportion of pups surviving until sampling.

## A) Rearing condition treated as a categorical variable

| | | rearing condition | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | (Intercept) | sol to com | purely com | com to sol | relocation | population size | age first | ls first | mean temp | df | AICc | ΔAICc | w |
| **Model 1** | **2.033** | + | | | **+** | **–** | **0.742** | **-0.475** | **–** | **9** | **981.6** | **0** | **0.383** |
| **Model 2** | **2.018** | + | | | **+** | **0.267** | **0.753** | **-0.477** | **–** | **10** | **982.5** | **0.90** | **0.244** |
| **Model 3** | **2.043** | + | | | **+** | **–** | **0.750** | **-0.476** | **-0.105** | **10** | **983.3** | **1.75** | **0.159** |
| **Model 4** | **2.052** | + | | | **+** | **0.331** | **0.764** | **-0.481** | **-0.160** | **11** | **983.8** | **2.22** | **0.126** |
| Model 5 | 1.903 | + | | | + | – | 0.814 | – | – | 8 | 987.1 | 5.48 | 0.025 |
| Model 6 | 1.890 | + | | | + | 0.256 | 0.826 | – | – | 9 | 988.0 | 6.43 | 0.015 |
| Model 7 | 1.912 | + | | | + | – | 0.821 | – | -0.94 | 9 | 988.9 | 7.29 | 0.010 |
| Model 8 | 1.898 | + | | | + | 0.316 | 0.837 | – | -0.149 | 10 | 989.4 | 7.83 | 0.008 |
| ... | | | | | | | | | | | | | |
| Model 64 | 1.440 | – | | | – | 0.392 | – | – | -0.132 | 5 | 1030.5 | 48.86 | 0.000 |
| Averaged parameters | | | | | | | | | | | | | |
| Estimate* | 2.03 | -0.66 | -0.62 | 0.02 | -0.56 | 0.12 | 0.75 | -0.48 | -0.04 | | | | |
| Unconditional SE | 0.20 | 0.22 | 0.21 | 0.28 | 0.17 | 0.21 | 0.18 | 0.17 | 0.11 | | | | |
| Lower 95%CI | 1.64 | *-1.10* | *-1.03* | -0.53 | -0.89 | -0.30 | *0.39* | *-0.82* | -0.26 | | | | |
| Upper 95%CI | 2.42 | *-0.23* | *-0.22* | 0.56 | -0.22 | 0.54 | *1.11* | *-0.13* | 0.18 | | | | |
| Relative importance | – | 1 | | | 1 | 0.41 | 1 | 1 | 0.31 | | | | |

The proportion of pups still alive at sampling was used as response variable in a GLMM with a logit link function. Models within 4 AICc units of the best model are highlighted in bold and were used to calculate averaged effect sizes (*standardised on two standard deviations following Gelman (2008)). The intercept represents a litter that was raised solitarily (solitary when first found and at sampling) and always found in the same nest box (not relocated). Female identity and the year in which a litter was born were used as random effects. Factors included are defined as follows. Rearing condition: the status a litter was first found and at sampling (abbreviations; com:communal, sol: solitary); relocation: a litter was relocated into a different nest box after found first (different nest box at sampling compared to first found); population size: the number of adults in the population during the month a litter was born; age first: a litter's age when first found [days]; ls first: a litter's size when first found; mean temp: the mean temperature in the barn during the month a litter was born).

## B) Rearing condition analysed as the number of additional litters joining a nest

| | (Intercept) | number of new litters | status first | relocation | population size | age first | ls first | mean temp | df | AICc | ΔAICc | w |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Model 1** | **1.753** | **-0.499** | **–** | **+** | **–** | **0.729** | **-0.415** | **–** | **7** | **984.4** | **0** | **0.200** |
| **Model 2** | **1.611** | **-0.488** | **+** | **+** | **–** | **0.716** | **-0.431** | **–** | **8** | **984.9** | **0.46** | **0.159** |
| **Model 3** | **1.742** | **-0.493** | **–** | **+** | **0.276** | **0.737** | **-0.416** | **–** | **8** | **958.3** | **0.87** | **0.130** |
| **Model 4** | **1.610** | **-0.484** | **+** | **+** | **0.250** | **0.724** | **-0.431** | **–** | **9** | **986.0** | **1.55** | **0.092** |
| **Model 5** | **1.756** | **-0.502** | **–** | **+** | **–** | **0.734** | **-0.415** | **-0.059** | **8** | **986.4** | **1.98** | **0.075** |
| **Model 6** | **1.611** | **-0.492** | **+** | **+** | **–** | **0.721** | **-0.432** | **-0.079** | **9** | **986.8** | **2.37** | **0.061** |
| **Model 7** | **1.745** | **-0.496** | **–** | **+** | **–** | **0.745** | **-0.416** | **-0.109** | **9** | **987.0** | **2.59** | **0.055** |
| **Model 8** | **1.608** | **-0.487** | **+** | **+** | **0.319** | **0.733** | **-0.432** | **-0.124** | **10** | **987.6** | **3.18** | **0.041** |
| **Model 9** | **1.678** | **-0.419** | **–** | **+** | **0.298** | **0.801** | **–** | **–** | **6** | **988.2** | **3.76** | **0.031** |
| Model 10 | 1.668 | -0.414 | – | + | – | 0.811 | – | – | 7 | 989.1 | 4.64 | 0.020 |
| Model 11 | 1.558 | -0.408 | + | + | 0.268 | 0.794 | – | – | 7 | 989.1 | 4.70 | 0.019 |
| ... | | | | | | | | | | | | |
| Model 128 | 1.328 | -0.141 | + | – | 0.368 | – | – | -0.141 | 6 | 1031.7 | 47.23 | 0.000 |
| Averaged parameters | | | | | | | | | | | | |
| Estimate* | 1.70 | -0.49 | 0.09 | -0.54 | 0.10 | 0.73 | -0.41 | -0.02 | | | | |
| Unconditional SE | 0.21 | 0.17 | 0.15 | 0.17 | 0.21 | 0.18 | 0.19 | 0.10 | | | | |
| Lower 95%CI | 1.28 | *-0.83* | -0.21 | *-0.88* | -0.30 | *0.38* | *-0.78* | -0.22 | | | | |
| Upper 95%CI | 2.10 | *-0.15* | 0.39 | *-0.21* | 0.51 | *1.08* | *-0.04* | 0.17 | | | | |
| Relative importance | – | 1 | 0.42 | 1 | 0.38 | 1 | 0.96 | 0.27 | | | | |

The proportion of pups still alive at sampling was used as response variable in a GLMM with a logit link function. Models within 4 AICc units of the best model are highlighted in bold and were used to calculate averaged effect sizes (*standardised on two standard deviations following Gelman (2008)). The intercept represents a litter that was found in a communal litter when first found and always stayed in the same nest box. Female identity and the year in which a litter was born were used as random effects. Factors included are defined as follows. Number of additional litters: the number of additional litters a focal litter was found with at sampling; status first: a litter's rearing condition when first found (solitary or communal); relocation: a litter was relocated into a different nest box after found first (different nest box at sampling compared to first found); population size: the number of adults in the population during the month a litter was born; age first: a litter's age when first found [days]; ls first: a litter's size when first found; mean temp: the mean temperature in the barn during the month a litter was born).

Table 12: Model summary statistics for the recruitment analysis

| | (Intercept) | status at sampling | population size | age at sampling | ls at sampling | mean temp | df | AICc | ΔAICc | w |
|---|---|---|---|---|---|---|---|---|---|---|
| **Model 1** | **-0.9175** | **–** | **0.5660** | **-0.2787** | **0.3066** | **-0.4248** | **7** | **1457.5** | **0** | **0.315** |
| **Model 2** | **-0.9155** | **–** | **0.5239** | **–** | **0.3262** | **-0.4338** | **6** | **1458.2** | **0.73** | **0.219** |
| **Model 3** | **-0.9461** | **+** | **0.5581** | **-0.2689** | **0.2794** | **-0.4253** | **8** | **1459.1** | **1.62** | **0.140** |
| **Model 4** | **-0.9505** | **+** | **0.5158** | **–** | **0.2928** | **-0.4339** | **7** | **1459.6** | **2.13** | **0.108** |
| **Model 5** | **-0.8402** | **–** | **0.5554** | **-0.3118** | **–** | **-0.4036** | **6** | **1461.4** | **3.98** | **0.043** |
| Model 6 | -0.9115 | + | 0.5412 | -0.2869 | – | -0.4086 | 7 | 1461.5 | 4.07 | 0.041 |
| Model 7 | 0.9143 | + | 0.4951 | – | – | -0.4174 | 6 | 1462.5 | 5.04 | 0.025 |
| Model 8 | -0.9075 | + | – | – | 0.3181 | -0.3691 | 5 | 1462.8 | 5.33 | 0.022 |
| Model 9 | -0.8325 | + | 0.5075 | – | – | -0.4174 | 5 | 1463.0 | 5.54 | 0.020 |
| ... | | | | | | | | | | |
| Model 32 | -0.9181 | + | – | – | – | – | 3 | 11473.5 | 16.06 | 0.000 |
| Averaged parameters | | | | | | | | | | |
| Estimate* | -0.92 | 0.03 | 0.55 | -0.17 | 0.29 | -0.43 | | | | |
| Unconditional SE | 0.17 | 0.09 | 0.20 | 0.19 | 0.14 | 0.12 | | | | |
| Lower 95%CI | -1.26 | -0.14 | *0.15* | -0.54 | *0.01* | *-0.67* | | | | |
| Upper 95%CI | -0.58 | 0.20 | *0.94* | 0.20 | *0.57* | *-0.18* | | | | |
| Relative importance | | 0.30 | 1 | 0.60 | 0.95 | 1 | | | | |

The proportion of sampled pups recruited into the population as tagged adults was used as response variable in a GLMM with a logit link function. Models within 4 AICc units of the best model are highlighted in bold and were used to calculate averaged effect sizes (*standardised on two standard deviations following Gelman (2008)). The intercept represents a litter that was found in a communal litter at sampling. Female identity and the year in which a litter was born were used as random effects. Factors included are defined as follows. Rearing condition: whether the litter was communal or solitary at sampling, population size: the number of adults in the population during the month a litter was born; age at sampling: a litter's age at sampling [days]; ls at sampling: a litter's size at sampling; mean temp: the mean temperature in the barn during the month a litter was born).

## DISCUSSION

The two alternative reproductive tactics of solitary or communal nursing proved not to be fixed over a female's lifetime. More than half (54.5%) of the reproducing females in our 5 year study of free-living house mice were observed to switch between solitary and communal nursing when they had at least 2 litters. Furthermore, the tactics did not result in equal fitness. The more litters a female raised solitarily, the higher was her reproductive success (measured as the number of offspring raised until 13 days of age during her lifespan in the barn). Pup survival was reduced in communal litters, while the mother's reproductive tactic did neither affect her likelihood to reproduce again nor her inter-birth interval. Our data suggest that communal nursing is a condition dependent alternative reproductive tactic. Female house mice live in a competitive social environment, characterised by high reproductive skew. Among those that managed to breed, it were the older females (likely of larger body weight) that raised a higher proportion of solitary litters and gained the highest reproductive success. The younger females, on the other hand, were more likely to use a "best of a bad job strategy" and communally rear litters even at the cost of reduced offspring survival. With increasing age and weight, they may improve their ability to nurse litters solitarily, given they survive.

### *Plastic alternative reproductive tactics*

Females displayed plasticity in their reproductive behaviour and switched between the two tactics. Population density and the season (analysed as the average temperature during the month a litter was raised) did not affect a litter's likelihood to be raised communally or solitarily. In general, more litters were raised communally (68.6% of all litters, see Table 6) than solitarily and the number increased over time. Older females were less likely to raise their litters as part of a communal litter.

Solitary litters could arise for different reasons. First, a female might have been the only female breeding in her social group without an option to nurse communally. Reproduction in the study population is seasonal and lowest during the coldest winter months (König and Lindholm, 2012). If older females were able to reproduce during this time of the year they would encounter few other breeding females. Nevertheless, since average monthly temperature did not affect a female's tactic, we reject that hypothesis. There is hardly any a priori reason to assume that older females should be more likely to give birth at times with no other breeding females around.

Second, females might not have found a social partner to their liking; therefore deciding against communal nursing. Weidt et al. (2014) showed in the same population (though a few years earlier and at a lower population density) that females were choosy and did not always communally nurse, even if other females in their group had dependent offspring at the time. The more partners a female had to choose from, the more likely she was to form a communal litter, indicating that females were selective in their choice (Weidt et al., 2014). This could also explain

why we observed an increase in the proportion of litters raised communally over the years, likely as a result of an increasing number of litters born (see Fig. 12) and therefore also partner litters to choose from.

It is still poorly understood how a communal litter is formed, especially in wild populations. In the laboratory, communal litters of 2 females occur when a highly pregnant female shares the nest with a partner already nursing a litter, and adds her pups to those present in the nest. In the free-living population, several females share several nest boxes (Weidt et al., 2014; König et al., 2015) and we assume that it is the female giving birth second who decides to join a litter for communal nursing. The already lactating female, on the other hand, may not necessarily agree with that decision and may have been joined against her interest. Such a situation may exist if younger females, of relatively low body weight, are not yet competitive in monopolising a nest box and thus avoiding exploitation by a non-preferred partner, as for example a female with a larger litter size. Once litters are pooled, females are unable to discriminate between own and other offspring, and forced to raise the entire communal litter, or to abandon the nest (König, 1989a).

Communal nesting has been described to happen preferentially among related individuals (Wilkinson and Baker, 1988), which would reduce the costs of potentially being exploited. It was shown both theoretically and empirically (in zebra finches) that individuals are more likely to tolerate exploitation by relatives, owing to indirect fitness benefits that compensate for at least some of the costs (Mathot and Giraldeau, 2010). In accordance with this, we found the pairwise relatedness (Wang estimator) between communally nursing females to be higher than the average relatedness among females in the population.

If females were tolerant towards exploitation by relatives we expected a high skew in litter sizes in communal nests (positive B index), suggesting that one exploits the partner(s) in term of their drastically increased investment in nursing other offspring. However, the variance in litters sizes observed within communal nests corresponded to that among all litters. Females therefore did not use infanticide to neither adjust a partner's litter size to her own litter size, nor to create a high skew. Nevertheless, females may still benefit unequally when raising a litter communally, given the variance in litter size.

A critical aspect here may be that we rarely know the size of a litter at its birth and therefore cannot analyse whether females giving birth second in a nest use infanticide to modify the skew already given by natural variance in litter sizes. A laboratory study on wild derived house mice, where litter sizes were known already at day of birth, did reveal no evidence that females use infanticide to equalise litter sizes, but they reduced the litter size of the female that had already given birth, but irrespective of their own litter size (Ferrari et al., 2016).

*Fitness consequences of communal nursing*

We observed a large skew in reproductive success (number of offspring weaned within lifespan in the barn) among the females in the study population. Approximately half of the adult females did not reproduce (none of their pups survived

until sampling). Such a finding indicates high reproductive competition among females (Clutton-Brock, 2009), one of the preconditions favouring the evolution of ARTs (Taborsky et al., 2008).

Our measure for LRT success was limited in two ways. First, we could not quantify the actual number of pups a female weaned (onset of weaning is at an age of 17 days), but instead used the number of pups sampled per female at 13 days of age. Younger pups are at a higher risk of being killed by conspecifics (Manning et al. (1995) never observed infanticide of pups older than 14 days in a semi-natural population), our measure therefore covered the period of highest risk, even though we slightly overestimated survival to weaning. Furthermore, we do not expect factors influencing pup survival from day 1 to 13 to differ from those impacting pups older than 13 days. While we could not measure the actual number of offspring weaned, we instead additionally analysed how many sampled pups were recruited into the population (caught and tagged as adults). Pups that disappeared between sampling and adulthood could either have died, or dispersed from the population. We observed that pups raised by mothers showing different reproductive tactics had the same probability of being recruited in the adult population. This indicates that solitary nursing will still be the superior behavioural option when using the number of recruited offspring into the population as a proxy for fitness.

The second aspect limiting our estimate of reproductive success was female lifespan. A large fraction of females in the data set ( 68.2%) were not found dead in the barn. Instead we only knew when they were last recorded alive in the population. Females that disappeared may have died and not been found in the barn, which we do not consider to happen frequently since we carefully check the barn during each population monitoring with hand-held readers for hidden tagged corpses. Alternatively, they may have died outside the barn or dispersed and left the population with unknown fate. We cannot exclude that some of the females survived and bred in another location, though dispersal likely is associated with high mortality and in many cases low reproductive success. We are nevertheless confident that our measure of lifespan and the number of offspring a female weaned represent a meaningful estimate of female reproductive success.

The strongest predictor of female reproductive success was - not unexpectedly - lifespan. The longer a female remained in the population, the higher the number of litters she raised and consequently the higher the number of pups reaching sampling age. More intriguingly, we found that with an increasing proportion of a female's litters being raised communally, her reproductive success was decreasing. Both population size and temperature during the time a females was born in had no negative effect on female reproductive success.

The reproductive tactic used by a female affected pup survival. Pups raised in communal litters had a higher mortality, which increased with each additional litter found together with the focal litter. Female infanticide seems the most likely explanation and has been described repeatedly for communally breeding female house mice (König, 1994a; Palanza et al., 2005; Schmidt et al., 2015; Ferrari et al., 2016). Females joining a nest with an already existing litter often kill one or several of the pups present, before they give birth themselves.

Decreased offspring survival in communal litters contradicts results from both experiments in the laboratory (König, 1994a) that found a higher reproductive success (measured over a lifespan of 6 months) for communally nursing females, and data from a semi-natural (Manning et al., 1995) and a free-living population (Auclair et al., 2014b), showing a higher survival for pups reared in communal litters. Both non-laboratory studies differed methodically from our study, because they analysed pup survival in communal nests after all females had pooled their litters. They thus likely missed infanticide occurring during or immediately before a new litter was added to a communal litter. This might explain the different results and indicate that females raising litters communally in a natural situation with high population density might be better at defending and caring for the joint litters. Nevertheless, this benefit did not outweigh the initial high costs of infanticide during the formation of a communal litter. The lifetime reproductive success of communally nursing females in a controlled laboratory setting on the other hand (König, 1994a) emphasises the importance of observations from free-living populations. Females in a laboratory setting experience a rather "simple" social environment, with only few, familiar group members, with low female competition (for food, nesting sites etc). We can not exclude that communal nursing at very low densities in the wild might have resulted in similar patterns.

*Condition dependent alternative reproductive tactics*

Female house mice used two alternative reproductive tactics and they switched from one to the other, indicating that it was a plastic tactic. One of the two tactics (communal nursing) resulted in reduced pup survival and consequently had a negative effect on female reproductive success. Given that communal nursing reduces female reproductive success, we would expect it to be absent or decreasing in frequency, however, this is not what we observed. Communal nursing even increased in our study population, has been described to occur both in the laboratory and in wild populations (Sayler and Salmon, 1971; Manning et al., 1995; König, 2006; Weidt et al., 2014), and is considered not to be a side-effect of group living (Weidt et al., 2014). All those findings suggest that the reproductive tactic of nursing litters communally is beneficial in specific situations.

The occurrence of two ART's with on average unequal fitness ouctcomes can best be explained by a condition dependent alternative reproductive tactic (Taborsky et al., 2008). Condition dependent ARTs are often characterised by individuals differing in their competitive abilities (Gross, 1996) and showing plasticity in choosing a tactic depending on their condition. Hence, we hypothesise that females might not always be able to rear their litters solitarily and instead opt for communal nursing, even at the cost of reduced pup survival. The alternative would be not to reproduce at all, or to delay reproduction which might be associated with even higher costs in a species experiencing low life expectancy (Manser et al., 2011). We find that age correlated with a female's likelihood to rear a litter communally, indicating that body condition indeed influenced the reproductive tactic of a female. Females increased in body weight when getting older (see Appendix D), so weight

and not age may have driven the effect. Heavier females are able to produce more milk (Ferrari et al., 2015) and likely are also better competitors in aggressive encounters. Body weight or body size were found to influence conditional dependent ARTs in many different species, both in males (Tomkins and Brown, 2004; Painting and Holwell, 2014) and in females (Hill et al., 2015b).

The higher proportion of solitary litters observed for older females allows for different interpretations. Older and likely heavier females were in better condition and might have afforded to raise their pups alone, therefore avoiding the costs. Younger females on the other hand, might not have been able to to efficiently protect their young against non-group members. Infanticide in mice, performed by both males and females if they encounter a nest with pups outside their own group, is common in wild populations (vom Saal, 1984; Manning et al., 1995; Auclair et al., 2014b) and reduces pup survival. Alternatively, younger females might have been unable to prevent other females from joining, therefore being forced to communally rear their litters. In accordance with our findings, free-living house mice were shown to communally nurse less often than we would expect based on empty and occupied nesting sites in their territory (unpublished data, *Harrison et al.*).

Female age proved to be an important factor influencing a female's reproductive tactic. External factors as the presence or condition of potential partner females nevertheless may further influence a female's tactic. For example, in a laboratory study, females were less likely to cooperate and raise their young communally if there was a high risk for exploitation,since females differed in litter size.

Communal nursing has been described for different rodent species and was often associated with reduced reproductive success for the females involved (Gerlach and Bartmann, 2002; Lacey, 2004; Hayes et al., 2009). Condition dependent ARTs can help to explain those findings and have been suggested to be of importance in striped mice (*Rhabdomys pumilio*) (Hill et al., 2015b). Analysing and interpreting an apparently cooperative behaviour as communal offspring care under the framework of condition dependent ARTs contributes to our understanding of how it is maintained in a population despite its fitness costs. In such a scenario, we would not expect selection for one or the other tactic, rather we would expect selection for the appropriate reaction norm, allowing individuals to choose the optimal tactic given the circumstances. Further research should therefore more strongly focus on deepening our understanding of the evolution of phenotypic plasticity and its role in cooperation.

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# ADDENDUM: METHODOLOGY

# 5

# A GENETIC TOOL TO MANIPULATE LITTER SIZE

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ABSTRACT

Experimental litter size manipulations are often not problem free. Typically conducted shortly after birth or oviposition, they do not account for the energy already invested into the production of the offspring. Such effects make it difficult to interpret the results from experimental litter size manipulations and therefore to study optimality of litter or clutch size, a long debated topic in evolutionary biology. We propose the use of a mating design based on a selfish genetic element, the *t* haplotype, to reduce litter size in an eutherian mammal, the house mouse. Most *t* haplotypes are recessive lethal and therefore lead to the death of all homozygous embryos. Litter sizes can be reduced by up to 50% by pairing a +/*t* female with a +/*t* male instead of a +/+ male. This method allows litter size manipulation before birth without the use of invasive techniques, therefore providing an excellent tool for studying optimal litter size and ultimately helping to understand life history strategies.

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KEYWORDS: Litter size manipulation, house mouse, *t* haplotype, optimal litter size

## INTRODUCTION

Reproduction is a key feature of life and ultimately determines the success of an individual. At any point in time an animal should therefore optimise its reproductive effort to maximise lifetime reproductive success. Several trade-offs play an important role in this process and determine to a large extent the life history of an animal. Pianka (1976) described the most important trade-offs with three simple, but crucial questions: when should an individual reproduce, how much should it invest into the current reproductive event and how much into one single offspring? The number of offspring produced by birds and mammals per reproductive event has been widely investigated over the last decades. Optimal litter or clutch size nevertheless remains puzzling as it is likely to be determined by the current environment, the trade-off between current and future reproductive efforts, as well as by the trade-off between the number and the quality of the offspring (Godfray et al., 1991).

*Testing optimality of litter or clutch size*

Already in the first half of the 20th century theories were developed to explain the huge variation observed in clutch size in birds. Lack (1947) proposed that survival probability decreases with increasing clutch or litter size, because the amount of food parents can provision to their offspring is limited. The "Lack clutch" is therefore defined as the clutch size which fledges the largest number of offspring. In the following years Lack's theory has been refined and the above mentioned trade-offs have been incorporated (Williams, 1966; Stearns, 1992).

The most common approach to test the assumptions of the "Lack clutch" or to investigate optimality of clutch or litter size is to manipulate the number of offspring to assess whether this reduces or increases the reproductive success of the parents and the offspring.

A variety of manipulative experiments have been conducted in birds, with contrasting results. For example, Styrsky et al. (2005) found that brood size enlargement in spotted antbirds (*Hylophylax naevioides*) increases juvenile mortality after fledging, whereas brood size reductions resulted in the opposite effect. Other studies found a delay of egg-laying and a decrease in the number of successfully reared young in the next brood for rooks (*Corvus frugilegus L.*) with experimentally enlarged broods (Roskaft, 1985).

A meta-analysis on 42 brood size manipulation experiments, on the other hand, found no evidence for the Lack hypotheses. Brood size enlargement did not lead to a reduction in the number of fledglings (Werf, 1992). Optimal litter size theory has also been applied to other vertebrates (mammals (Sikes and Ylönen, 1998); reptiles (Aubret et al., 2003)) and invertebrates (Hardy et al., 1992).

In mammals, litter size manipulations are usually conducted shortly after birth by adding or removing pups of similar age. Such manipulations affected the growth rate of offspring in rodents (white-footed mice (*Peromyscus leucopus*) (Fleming and Rauscher, 1978), wild bank voles (*Myodes glareolus*) (Koskela, 1998; Mappes et al., 1995)) and the future reproductive success of females and their daughters (house mice (*Mus musculus domestics*) (Fuchs, 1982)). Other studies, in contrast, did not observe an effect of litter size manipulation on offspring condition (ground squirrels (*Spermophilus columbianus*) (Hare and Murie, 1992)) or female future reproduction (wild bank voles (Koskela, 1998; Mappes et al., 1995), ground squirrels (Hare and Murie, 1992)). Correlational data also suggests that there is no such trade-off (northern grasshopper mice (*Onychomys leucogaster*) (Sikes, 1998)). This discrepancy between different studies and methods (observational, versus experimental litter size manipulations) may indicate that postpartum manipulation of offspring number does not reflect a "naturally" large or small litter size. If females give birth to a litter size that is optimised to their current physiology and condition, manipulation of number of pups directly after birth will not result in standardization of lactational burden for different females (for a review see Jameson (1998), and next section).

*Problems associated with experimental litter size manipulations*

One main problem of clutch or litter size manipulation experiments is that they do not account for the energy already invested into the production of the offspring.

The cost of egg production and incubation in birds was largely ignored, until Monaghan et al. (1998) showed that it can have a substantial effect and should not be overlooked. In altricial house mice, energy demand increases during gestation by 49.2% (compared to nonreproducing females (Speakman and McQueenie, 1996)). Such an increase is substantial, although lactation comes at even higher costs (house mice (Speakman and McQueenie, 1996), bank voles (Migula, 1969)).

Pregnancy in eutherian mammals further differs from the pre-incubating phase in egg-laying birds by its effect on the mother's hormones and behaviour. Mammary development begins already during gestation, and *in utero* litter size directly affects hormone levels (goats (Hayden et al., 1980, 1979; Manalu et al., 1997), mice (Soares and Talamantes, 1983)), mammary gland size (sheep (Rattray et al., 1974), goats (Hayden et al., 1980), mice (Knight, 1982)) and therefore likely also milk yield after birth (goats (Hayden et al., 1979)). In addition, body weight of pregnant females increases with increasing prepartum litter size or litter mass (Duah et al., 2013). Such an effect may have consequences for later lactation since heavier females produce more milk than smaller ones (König et al., 1988).

Despite the influence of *in utero* number of pups on maternal physiology and behaviour, adjustment to modified postpartum litter size is possible. Experimental litter size manipulation after birth revealed compensatory mammary growth in the first days of lactation, suggesting an ability to adjust milk production to changing litter sizes after birth (Knight, 1982; König et al., 1988; Duah et al., 2013). Nevertheless, to what extent pre- and postpartum litter sizes influence maternal behaviour and lactation remains controversial. Analysing that question requires methods to manipulate litter size during gestation. One option is to surgically remove embryos at an early stage of the pregnancy (house mice (Nagasawa and Yanai, 1971)). This surgical method, however, is very invasive and the effects of the surgery difficult to control. Similar problems could arise after the removal of one of the ovaries prior to breeding. This method has been used in pigs to reduce litter size (Kensinger et al., 1986).

*The t haplotype as a tool to manipulate litter size*

As an alternative, we propose here to use the *t* haplotype as a genetic tool to reduce litter size in an eutherian mammal, the house mouse, which is widely used as a laboratory animal. This method allows for a predictable noninvasive litter size reduction without postnatal interference. The *t* haplotype is a selfish genetic element occurring in natural house mouse populations (for a review see Silver (1993)). It is located on chromosome 17 and consists of four linked inversions, spanning approximately one third of the whole chromosome (Silver, 1993). The *t* haplotype has been described for all four subspecies of the house mouse (*Mus m. domesticus, Mus m. musculus, Mus m. castaneus and Mus m. bactrianus*)(Hammer

et al., 1989; Silver, 1993). Gene products of the *t* haplotype affect the development of the flagella of wild type sperm during spermatogenesis in +/*t* males, leading to a transmission ratio distortion with a *t* gamete transmission of up to 99% to the offspring (Silver, 1993). In females *t* gamete transmission follows the classical Mendelian rule with on average 50% of the gametes receiving the *t* haplotype. By amplifying and scoring a genetic marker (*Hba-ps4*) associated with the *t* haplotype (Hammer et al., 1989), this selfish genetic element can easily be identified. The *t* can be found in many wild populations and several different *t* variants are commercially available (to give an example: mouse strains $t^{w5}$ (RBRC01202) and $t^{w5G}$ (RBRC01203) from the Experimental Animal Division of the RIKEN BioResource Center). Because of the transmission ratio distortion in males, the *t* can be crossed into a population or specific strain within a rather short time (see Lenington et al. (1994)).

Most of the different *t* variants carry recessive lethals, causing the death of homozygous individuals *in utero*. The stage in which lethality occurs varies between *t* variants, but most often it happens around day 9 or 10 of pregnancy (Safronova, 2009). At this stage embryos are typically between 1.2 mm [day 9] and 3.9 mm [day 10] in size (Theiler, 1989). Nagasawa and Yanai (1971) surgically adjusted the number of foetuses at day 8 of pregnancy in mice. They sacrificed the females at day 19 of pregnancy and analysed mammary development. The indices used to measure mammary development correlated positively with the number of embryos left after surgery suggesting that prepartum litter size (after day 8 of pregnancy) quantitatively influenced the development of the mammary gland tissue. The litter size reduction due to the recessive lethal nature of the *t* haplotype, acting in the first half of the gestation period as described above, should therefore still allow for adjustment of prenatal mammogenesis to the number of surviving embryos. It is exactly the recessive lethal property of the *t* haplotype that can be used as an instrument to reduce litter size, without interfering after birth, or applying invasive surgery to remove foetuses.

## RESULTS AND DISCUSSION

Under standardised laboratory conditions, we analysed litter sizes at birth from four different mating crosses of +/*t* and +/+ house mice, originating from a wild population. A significant litter size reduction was observed when +/*t* females were mated with +/*t* males ($F_{3,123}$=86.79, p-value<0.001)(Lindholm et al., 2013). Model estimates of the mean are displayed in Fig. 17. The litter size at birth of +/*t* females mated with +/*t* males was approximately 40% smaller than the litter size of any other mating cross (Fig. 17, (Lindholm et al., 2013)).

Like all methods, this genetic tool comes with some limitations. Manipulation is only possible in one direction. Litter size can only be reduced, but not increased. Increasing litter size requires another method. Currently, litter size can be increased prepartum by inducing superovulation with gonadotrophins (house mice (Wilson and Edwards, 1963), sheep (Newton et al., 1970), bank voles (Oksanen et al., 2002)).

Furthermore, based on the mating design required for reduced litter sizes at birth, females with small and standard litters will either differ in their genotype (+/*t* or +/+) or the genotype (+/*t* or +/+) of the sire of their litter, or both. It is therefore not possible to completely disentangle other effects of the *t* besides the reduction in litter size.

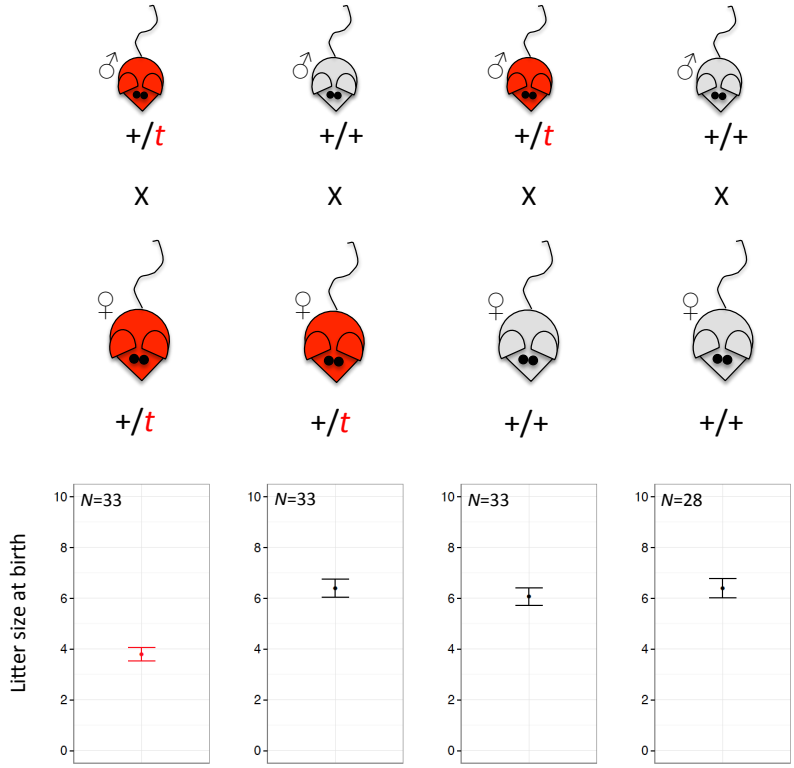


Figure 17: Litter sizes at birth for all four different mating crosses between +/t and +/+ mice are displayed. Plotted are back transformed model estimates [means] (glm) and the standard error of the mean.

The *t* is known to affect functional sperm in males and behavioural studies have revealed that +/*t* females prefer +/+ males over +/*t* males, probably to avoid a reduction in litter size (Lenington et al., 1992; Lindholm et al., 2013). In the population from which our experimental animals derived, the *t* is associated with a unique MHC haplotype, and could thus play a role in *t* dependent mate choice (Lindholm et al., 2013). Nevertheless, we do not expect mate choice to be a confounding factor in the setting presented here. First, litter size manipulation experiments are typically conducted in the laboratory and females are paired monogamously with males. In our laboratory crossings, both +/+ and +/*t* females did not differ in their propensity to conceive and to give birth when mated with +/*t* compared to +/+ males (Lindholm et al., 2013), indicating that they did not discriminate against +/*t* males. Second, the majority of experiments using litter size

manipulations in house mice focus on the behaviour of the dams and /or the offspring, and there are up to now no indications that the *t* directly influences maternal behaviour.

A rather simple experimental design could thus help to answer to what extent prepartum versus postpartum number of offspring influences female reproductive costs, physiology and behaviour, by combining the genetic method to manipulate litter size prenatally with manipulations of litter size at birth. Furthermore, +/t females can alternatively be paired with a +/*t* male and with a +/+ male (full-sibs if required), or vice versa, therefore making it possible to compare data from the same female, once with a reduced and then with a standard litter size. In addition litter size reductions could help to reduce the number of mice born during experiments (in line with the 3R recommendations (Russell and Burch, 1959)).

## CONCLUSIONS

Experimental litter or clutch size manipulations are an important tool for gaining insight into the optimal litter size, and ultimately to understand life history strategies. Such manipulations can however cause substantial problems whenever the energy invested into the production of eggs or into gestation is ignored. Using a recessive lethal gene can help to reduce litters or clutches in a predictive way without interference after birth or oviposition.

Recessive lethals can only generate litters that are reduced on average by 25%, but the transmission ratio distortion caused by the *t* haplotype in male house mice results in a litter size reduction of up to 50%. The earlier the recessive lethal property of the gene works, the better it controls for the prenatal costs of reproduction and potential prenatal adjustments to the litter size. Selfish genetic elements are assumed to be wide spread and often associated with recessive lethals, therefore similar methods could apply for a whole array of species (Burt and Trivers, 2006). This novel method allows the generation of smaller litters in a mammalian species without interfering after birth or using invasive techniques.

## MATERIALS AND METHODS

The data presented in this study were collected as part of a larger data set (Lindholm et al., 2013). Data from experiment 1 and 2 of (Lindholm et al., 2013) were pooled for this analysis. In short, mice used were F1 to F3 descendants from wild house mice caught between 2006 and 2008 at a study population in Illnau, near Zurich, Switzerland. For more details on the free living study population see Lindholm et al. (2013); König and Lindholm (2012). Experiments were conducted in an animal facility at the University of Zurich. Prior to the experiments mice were kept in same-sex sibling groups after they had been removed from their parental cage at an age of 28 days. At that point a tissue sample was taken from each mouse for genotyping. The *t* haplotype was identified by scoring the genotype at the *Hbaps4* locus (Hammer et al., 1989)(for a detailed method see (Lindholm et al., 2013)). Mice used in the experiment inherited the *t* from the paternal, or maternal side.

For simplicity, we always refer with $+/t$ to heterozygous indivudals, irrespective of whether they inherited the $t$ from their mother or father. To our knowledge there are no imprinting effects known for the $t$ haplotype.

During the experiments a male and a virgin female were kept together in a Macrolon type III cage (425mm x 266mm x 155mm). The male was removed from the cage after 14 days and from day 19 onwards, cages were checked daily for new litters. After birth cages were searched for living and dead pups. All possible combinations of crosses between $+/t$ and $+/+$ mice were used. In total 127 mating crosses were analysed. The exact numbers of each combination are indicated in Fig. 17.

*Statistical analysis*

All analyses were performed with R 2.15.1 (R Core Team, 2015). A generalised linear model (glm) was used to test for an effect of the four different mating crosses on the litter size. The glm was fitted using a quasipoisson error distribution with a log-link function. Significance was tested by conducting F-tests, alpha was taken to be 0.05.

## AUTHOR'S CONTRIBUTIONS

Manuela Ferrari confirmed the method in Chapters 1, 2 and wrote the main text. Anna Lindholm conceived the methodological idea, performed the mating crosses and contributed to the text. Barbara König supervised the work and contributed to the text. All authors read and approved the final manuscript.

## ACKNOWLEDGEMENTS

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# GENERAL DISCUSSION

# GENERAL DISCUSSION

The findings from both laboratory experiments and data from a wild house mouse population presented in this thesis can be discussed under two aspects that are fundamental for understanding the evolution and maintenance of cooperation in this species.

First, I will discuss whether there is potential for exploitation among communally nursing females and what might be the mechanisms that stabilise cooperation. Analogue to the framework developed in the General Introduction, I will focus on female investment and the number of offspring a female contributes to the nest.

Second, communal nursing needs to be assessed as one possible reproductive strategy out of two alternatives. Females in the wild use both tactics (Weidt et al., 2014) and we cannot analyse one, without taking into account the alternative. In the second part of this discussion I will therefore concentrate on the fitness consequences of communal nursing in free-living house mice and speculate about the factors maintaining it in a population exposed to natural selection.

Lastly, I will combine the findings discussed in both parts and draw some final conclusions. With only a few experiments and data from one single population, some of my conclusions will necessarily be speculative. I nevertheless hope that they might help us to get a better insight into communal breeding and provide food for thought for future research.

## CONFLICT POTENTIAL AND THE RISK FOR EXPLOITATION

Chapter 1, 2 and 3 focused on analysing the potential of conflict among reproducing female house mice by conducting experiments in the laboratory. Females were shown to invest according to the total number of pups in the nest, and not their own litter size, making them highly vulnerable to exploitation (Chapter 1) and indicating that they indeed did not discriminate between own and alien offspring (in agreement with König (1989a)). Whenever females differed in the amount of pups they had in the nest, they risked to overinvest, or in case of the female with the relatively larger contribution, they gained a benefit by underinvesting. Chapter 1 and 2 further confirmed that females often differed in their litter size, on the one hand because of differences in birth litter sizes, and on the other hand because females practiced infanticide and gained an advantage by killing some of the other female's offspring (Chapter 2). Both of these aspects and their impact on cooperation in communally breeding species will be discussed in the following.

A female's apparent inability to recognise her own young exposes her to the risk of being exploited. Such an inability has been described for a number of communally breeding species (Mumme R. L. et al., 1983; Johnstone and Cant, 1999; Bourke, 1994). The evolution of the ability to discriminate between own and other

offspring would convey an advantage to females in the communal nursing setting, allowing them to benefit at their partners cost. First, they could preferentially nurse own young and, secondly, they could reduce the other female's litter size up to the point where they could kill all young of other females, therefore directing all the maternal care towards their own offspring. Such behaviours would likely jeopardise the evolution of cooperation.

Still, it is puzzling why females do not discriminate between own and others. Female house mice adjust maternal behaviour to the developmental stage of their litter (König and Markl, 1987), illustrating that they have information on specific traits of their pups. We thus may expect that females use such information, and to do so they only require information on whether they gave birth to the younger or to the older pups in the communal litter. However, the risk of accidentally discriminating against own offspring may be too high for a female to rely on such traits. We do not know therefore, whether females are unable to recognise their own young, or whether they do not distinguish because the costs associated with making mistakes might be too high.

In a way, it is the inability to recognise own young that facilitates communal nursing, because it prevents females from only caring for their own young and/or from killing alien offspring in the nest, which likely would result in the collapse of the cooperation. It thereby grants females the possibility to benefit from the potential benefits associated with communal offspring care such as increased protection against infanticide from non-group members (Manning et al., 1995), an improved pup immune response due to more various antibodies and immunoglobulins received through milk from several females (Roulin and Heeb, 1999), by allowing females to spend less time in the nest (Auclair et al., 2014a) and improved thermoregulation (Hayes, 2000).

Female-offspring conflict could be one of the reasons why mothers are not able to recognise their own young. Parents and offspring are in conflict over how much energy they should invest into the current vs. future reproductive events, with offspring favouring increased current investment because they are more closely related to themselves than even to their potential fullsiblings produced in their mother's next litter (Trivers, 1974). Translated into the communal nursing context, females should have an interest to preferentially care for their own young, while the offspring also benefits from care received from other females. A female's inability to recognise own from alien offspring therefore is in the interest of young in communal nests, since it protects them from infanticide (through maternity confusion) and secures them the care of more than one female.

Similarly, it also has been argued that sexual conflict between males and females could result in a similar phenomenon. Males and females have conflicting interests when it comes to the amount of energy a female should invest into the offspring, with the optimal investment for females being lower than what males would favour, as long as they are not monogamous over several breeding attempts (Houston and Davies, 1985). Consequently, males mating with several females contributing to one communal nest and therefore siring most of the offspring, have an interest that all young are cared for, irrespective of relative female contributions.

Male imprinting has therefore been suggested as one way how the evolution of a recognition mechanisms could be prevented in communally breeding species (Roulin and Hager, 2003). Recent studies, however, revealed that offspring raised in communal litters often had been sired by different fathers (Auclair et al., 2014b), speaking against the paternal imprinting hypothesis in house mice.

Given that females cannot tell between own and alien offspring in the same nest, how could they minimise the risk of exploitation by their partner? One option to do so would be to lower overall investment, as it was observed in several birds species, with males reducing feeding rates with increasing rates of extra-pair matings by their partner (i.e. offspring in the nest that were sired by different males) (Burke et al., 1989; Chuang-Dobbs et al., 2001; Rios-Cardenas and Webster, 2005). Chapter 1 showed that communally nursing females that had relatively fewer offspring in the nest overinvested compared to solitarily nursing females, even though they reduced the energy content of their milk (lower lipids) likely as a measure to compensate the slight overproduction of milk. Females might therefore be constrained in their ability to reduce milk production, because the amount is determined by the number of suckling young (Chapter 1; (König et al., 1988; Duah et al., 2013)). Alternatively, females might be prevented from reducing their investment through aggression by their partner (Chapter 3). However, I found no evidence for punishment among communally nursing females, again indicating that females do not reduce investment as a measure to reduce the risk of being exploited (Chapter 3).

Females not discriminating between own and alien offspring also influences the patterns of infanticide we see among communally nursing females. Infanticide in the laboratory was only observed before both females gave birth. If females cannot tell between own and alien offspring in the same nest, a still pregnant female committing infanticide will not risk retaliation by the partner who gave birth first, after delivering her own litter. Females should therefore try to avoid giving birth first, which consequently might select for a synchronisation of birth or egg laying, as indeed has been observed in a number of species (Hodge et al., 2011; Riehl, 2016). If all females gave birth at the same time, the risk of accidentally killing own young would be too high, making the behaviour unfavourable. However, birth synchronisation would not eliminate the increased costs of investment when females have relatively fewer offspring in the nest than their partners.

The female's inability to recognise her own young, together with limited options to reduce overall investment, might therefore constrain a female's options to avoid exploitation after the formation of the communal nest. Selection may rather favour mechanisms that act before communal nursing begins. Social partner choice, or generally being selective about with whom to communally rear offspring, should therefore be important. There is evidence that females are choosy (Weidt et al., 2007, 2014) and some results provided in this thesis further emphasise the importance of the decision whether and with whom to communally nurse (Chapters 2 and 3). Females were found to be aggressive towards unfamiliar and unrelated females, with only ~50% of the initial female pairs succeeding in rearing a communal litter, the remainder of the pairs had to be separated due to fighting

(Chapter 3). Even more convincingly, Chapter 2 showed that females were less cooperative, showing a lower probability to engage in communal nursing, if the potential for conflict (the difference in litters size between females) was large, even among full sisters. Both these results indicate that deciding whether to communally nurse or not in a given situation is of fundamental importance, likely being the only way that females can reduce the risk of being exploited.

### NEGATIVE FITNESS CONSEQUENCES OF COMMUNAL NURSING IN THE WILD

in Chapter 4 I analysed female reproductive success in a wild population in relation to whether females reared their young solitarily or communally. Contradictory to what was expected based on laboratory experiments (König, 1994a), we found no evidence that communal nursing improves female success. On the contrary, communal nursing negatively impacted pup survival and resulted in a lower reproductive success for females that raised a larger proportion of their litters communally. Infanticide commited by the other females seemed the most likely cause of this reduction in pup survival based on the findings in Chapter 2 and in agreement with the literature (König, 1994a; Palanza et al., 2005; Schmidt et al., 2015). Overall, if we compare survival data from the laboratory (Chapter 2, Fig. 8) and the wild population (Chapter 4, Fig. 16), they are remarkably similar. In the laboratory, females were found to kill some pups before they gave birth themselves (first born litters had a lower survival probability) and in the wild population, pup survival equally decreased with each new litter joining a nest. However, in contrary to the laboratory, where the female giving birth second did not suffer a reduction in pup survival, females in the barn seemed to have a disadvantage when communally rearing their young, irrespective of the order. I was not able to analyse order directly due to methodological limitations, but I found no evidence for reproductive skew among females during communal nursing, as expected if the litter of the last female to join would not suffer from increased mortality. I cannot exclude, however, that order in the barn still does play role and we would see a slightly higher survival for pups of the last female to join, if the data would have allowed to incorporate litter size at birth and when females were joining the communal nest. It would not change the finding though, that on average, communally nursing females suffered from lower survival of their own pups compared to solitarily nursing females.

The difference in survival between litters reared solitary and communally was rather small (see Fig. 15 B), but it nevertheless raises the question why communal nursing still exists in the wild population? If solitary nursing is beneficial, selection should favour females rearing their litters alone, which is not what we observed in our wild population, with approximately 70% of all litters being sampled as part of a communal litter. The occurrence of two discrete reproductive tactics, that differ in fitness is difficult to explain under a framework in which communal nursing was selected as an adaptive behaviour. We would at least expect equal mean fitness for the two tactics for them to be evolutionarily stable and maintained in the population.

Negative effects of communal breeding, however, were not only described for house mice, but for a number of mammals (Gerlach and Bartmann, 2002; Lacey, 2004; Hayes et al., 2009). Rearing young communally might therefore represent a "best-of-a-bad-job" situation, meaning that while communally nursing females suffer some costs, it still is the best they can do given their situation. Females not in the best condition might be unable to rear a litter solitarily, leaving them with the options either not to breed at all, or to rear a litter communally. If the decision is linked to female condition (as age or weight), this would represent a condition dependent alternative reproductive tactic (Gross, 1996). A condition dependent alternative reproductive tactic, as supported by the results of older (and likely heavier) females rearing litters more often solitarily (Chapter 4), would allow for both tactics to be observed in the population, even though the average fitness is lower for communal than for solitarily breeding.

Likewise, conditions not related to a female's body status, but her environment, could lead to similar results. If nesting sites were limited, females might equally be presented with the option to communally rear their young, to abstaining from reproduction for the time being or to disperse.

## CONCLUDING REMARKS

Communal nursing in house mice provides potential for exploitation, because females invested according to the total number of pups in the nest and not their own litter size (Chapter 1). The high rate of female infanticide (Chapter 2) and aggression before the onset of reproduction (Chapter 3) further emphasise pronounced reproductive competition. Females seemed to have only limited options to avoid exploitation after the formation of the communal nest, which is expected to select for decision making in whether, or with whom to communally nurse.

High reproductive competition among communally nursing females resulted in lower pup survival in a wild population (Chapter 4), indicating that in many instances the behaviour represents a "best-of-a-bad-job" tactic. Only older females, presumably heavier and in good condition might be able to raise a litter solitarily, all other females have to opt for communal nursing even at the cost of a reduction in pup survival, or not to reproduce at all. Nevertheless, females might minimise the costs by carefully choosing with whom to cooperate. The negative fitness consequences are therefore not necessarily contradictory to the findings in the laboratory or the literature, showing that females avoid situations of high conflict (Chapter 2) or are selective in with whom to cooperate (Weidt et al., 2007, 2014).

Little is know about the formation of a communal nest, which complicates the interpretation. Are all partners involved in the decision to set up a communal litter, or are some simply unable to prevent others from joining? Given the female's inability to recognise own from other offspring, the moment litters are pooled, females can only abandon their litter or care for all offspring in the communal nest. The finding that communally nursing females were more closely related to each other than corresponds to the average degree of relatedness among females

(Chapter 4) suggests that the female that is joined had some control over who she was accepting. Mathot and Giraldeau (2010) showed that individuals should be more likely to accept related individuals exploiting them, than unrelated ones. However, if the females that gave birth first are unable to prevent others from joining, the situation rather resembles a parasitic tactic. While the females that join the nest still provide some care - in contrast to classical examples of brood parasitismus, with individuals providing no care at all - they can direct a larger proportion of the maternal care towards their own young, by reducing the other female's litter size before giving birth themselves, therefore exploiting their social partner (Chapters 1 and 2). Given the high costs of lactation in mice and that communally nursing females are often related to each other, we would, however, not expect the evolution of conspecific brood parasitism (Zink, 2000). Individuals would benefit the most from parasitising unrelated females and the high costs of raising additional young likely would reduce the survival of all pups in the parasitised nest, also resulting in reduced success for the parasitic female (Zink, 2000). Further research is needed to determine the role the partners play when establishing a communal nest.

The data presented in Chapter 4 originates from one population that is characterised by a rather high population density throughout the years analysed. It might be, that at a lower population density, and likely lower survival probability and reduced reproductive competition, the fitness consequences of communal breeding would be different, more resembling the findings from the laboratory (König, 1994a). The occurrence of predominantly small communal nests (with only 2 partners) might be more common at lower population densities. Such a situation is not as detrimental to pup survival as nests consisting of 3, 4 or more females (see Chapter 4, Fig. 16). It would be interesting to look at different populations and or population densities to asses to what extent this might influence the benefits and costs of communal nursing.

To conclude, based on the findings presented in this thesis, a female should, preferentially rear her pups solitarily. If weight (which might be age related), or the social circumstances, do not allow her to monopolise a nesting site, the next best option would be to join an already existing litter. Two females might then be more likely to be able to fend off further females from joining. Even, if this is not possible, the costs of infanticide would at least be shared by the litters already present in the communal nest.

As mentioned before, communal breeding has been associated with costs in a variety of species. In those species, the per capita success per female decreased with increasing number of females contributing to the communal nest, analogue to what I found in Chapter 4. Condition dependency might therefore play a role in many facultative communally breeding species and has already been described for African striped mice (*Rhabdomys pumilio* (Hill et al., 2015b,a)).

The results of this thesis emphasise the importance of analysing the potential of conflict in an apparent cooperative behaviour as communal nursing. Only by understanding the risks involved in all aspects of the cooperation, and by including all possible alternatives a female may have, as solitary nursing in the case

of female house mice, its evolution as well as maintenance in a population can be understood. Furthermore the results reveal that an apparent cooperative behaviour does not necessarily always result in benefits for all individuals involved. Plastic and condition dependent alternative reproductive tactics could help to explain how such behaviours still can be maintained in populations, despite their fitness costs, highlighting the importance for future research about the evolution of plasticity and its effect on cooperation.

# APPENDIX

# A

## APPENDIX TO CHAPTER 1

### PUP BODY WEIGHT

Table 13: A total of 1655 body weight measures were used for analysis. The exact number of data points for all age classes [days] and treatments are displayed. The day of birth corresponds to day 1, weaning to day 23, and pups were removed from the parental cage with 28 days.

| **pup age** [days] | **solitarily reared** [N] | **communally reared** [N] |
|---|---|---|
| **1** | 132 | 97 |
| *(2)* | 20 | - |
| **5** | 90 | 156 |
| *(6)* | 20 | - |
| **9** | 90 | 161 |
| **13** | 101 | 169 |
| **17** | 100 | 152 |
| **23** | 81 | 153 |
| **28** | 70 | 63 |

### GENOTYPING AND PARENTAGE ANALYSIS

The *HbA-ps4* marker was used to identify the genotype at the *t* locus (+/+ or +/*t*) (Hammer et al., 1989). We used 12 markers for the parentage analysis of pups that could not be assigned to a mother (markers: Chr1_20, Chr5_20, D7Mit319, Chr1_11, Chr12_2, X3, X57, oxt-ms, D3Mit278, D6Mit139, Chr8_3, Chr19_17, D11Mit90) (Bult et al., 2007; Teschke et al., 2008). Pups were assigned to one of the females by manually comparing the alleles of the 12 markers, allowing for one mismatch between mother and offspring.

# B

## APPENDIX TO CHAPTER 2

### HOW TO ASSESS SIGNIFICANCE OF FIXED EFFECTS IN MIXED MODELS

The model without the factor of interest, fitted to the data, was used to simulate data. This simulated data was fitted to the reduced model and the full model containing the factor of interest. The difference in deviance between the models was calculated to get an estimate for how well the full model would fit the data, even if the additional factor had no effect (the whole process was iterated 10000 times). The distribution of differences in deviance was than compared to the actual difference we can observe between the reduced and full model when fitted to the actual data. The comparison of the difference in deviance was done with a $\chi^2$ test.

R code:

```
modelCompare ← function(model1, modelo, niter=10000){
if(isREML(model1)) model1 ← update(model1, REML=FALSE)
if(isREML(modelo)) modelo ← update(modelo, REML=FALSE)
obs.chisq ← deviance(modelo) - deviance(model1)
sim.chisq ← replicate(niter,
{
newresp ← simulate(modelo)
nmodo ← refit(modelo, newresp)
nmod1 ← refit(model1, newresp)
deviance(nmodo)-deviance(nmod1)
})
p ← (1+sum(sim.chisq >obs.chisq))/(niter+1)
return(list(chisq=obs.chisq, p=p))}
```

### BINOMIAL GLMM: BACKTRANSFORMATION TO OBTAIN AN ESTIMATE OF THE MEAN

The following formula, described by Hadfield (2013), was used to backtransform the model estimates from a binomial GLMM to receive an approximation for the mean and the confidence interval around the mean.

$$c2 = (\frac{16*\sqrt{3}}{(15*\pi)})^2$$

$$\text{plogis}(\frac{\text{model estimate}}{\sqrt{1+c2*\text{ variance explained through random factors}}})$$

### LITTER SIZE MANIPULATIONS

Litter size differences in laboratory studies are usually generated by reducing or increasing the number of pups after birth (König et al., 1988; Hammond and Diamond, 1992). This approach raises problems that interfere with the questions we aim to answer (Ferrari et al., 2014). That is why we decided instead to use the *t* haplotype to generate an asymmetry in litter size. The *t* haplotype is a selfish genetic element, occurring in wild house mouse populations (for a review see Silver (1993) ). This selfish genetic element is characterised through drive in males (up to 90% of a $+/t$ male's offspring inherit the *t*) and is often typically associated with recessive lethals, leading to the death of all homozygous embryos already in utero between day 1 and 10 of pregnancy (Safronova, 2009). Females carrying the *t* haplotype therefore suffer on average from a litter size reduction of up to 40% when they mate with a $+/t$ male (Lindholm et al., 2013). In our experiment we used sister pairs, with one female being $+/t$ and the other +/+, which allowed us to create a difference in litter size between the females by pairing them with a $+/t$ male in the experimental treatment. In the control treatment females were paired with a +/+ male and as a consequence we expected no difference in litter size.

### GENOTYPING AND PARENTAGE ANALYSIS

Tissue samples from experimental animals were taken at weaning, prior to the onset of the experiment. The *HbA-ps4* marker was used to identify the genotype at the *t* haplotype (+/+ or $+/t$ ) (Hammer et al., 1989). Twelve microsatellite markers were used for the parentage analysis of pups that could not be assigned to a mother, (markers: Chr1_20, Chr5_20, D7Mit319, Chr1_11, Chr12_2, X3, X57, oxtms, D3Mit278, D6Mit139, Chr8_3, Chr19_17, D11Mit90) (Bult et al., 2007; Teschke et al., 2008). Pups were assigned to one of the females by manually comparing the alleles of the 12 markers, allowing for one mismatch between mother and offspring. In four social groups with litters born at the same day a total of 18 pups disappeared (were eaten completely) before a sample was taken. It was therefore not possible to collect tissue samples and assign them to a mother.

# C

# APPENDIX TO CHAPTER 3

## DETAILS ABOUT THE FACTOR ANALYSIS

Table 14: Summary of exploratory factor analysis results, using Maximum Likelihood Estimation (N = 174)

| | Factor loadings | |
|---|---|---|
| factors | Socio-negative behaviours (1) | Socio-positive behaviours (2) |
| resting with body contact | -0.05 | -0.25 |
| grooming | -0.07 | **0.33** |
| chasing | **0.99** | 0.05 |
| biting | **0.99** | 0.07 |
| sniffing at nose | 0.09 | **0.71** |
| sniffing anogenital area | **0.46** | **0.63** |

## CORRELATION MATRIX OF THE SIX BEHAVIOURAL TRAITS MEASURED

Table 15: Correlation matrix of the six behavioural traits used in the factor analysis. (N = 174)

| | resting with body contact | sniffing at nose | sniffing at anogenital area | chasing | biting | allo-grooming |
|---|---|---|---|---|---|---|
| resting with body contact | 1.0 | -0.23 | -0.13 | -0.06 | -0.07 | -0.08 |
| sniffing at nose | – | 1.0 | 0.49 | 0.12 | 0.14 | 0.21 |
| sniffing at anogenital area | – | – | 1.0 | 0.49 | 0.51 | 0.20 |
| chasing | – | – | – | 1.0 | 0.99 | -0.04 |
| biting | – | – | – | – | 1.0 | -0.04 |
| allogrooming | – | – | – | – | – | 1.0 |

# D

## APPENDIX TO CHAPTER 4

*Female body weight*

We analysed the effect of female age on body weight using a linear mixed model. For 252 females that gave birth between 2007 and 2011 we had between 1 and 13 measures of their body weight at times they were not pregnant (determined visually). The mean number of weights per female was 4.17. Female age had a strong effect on weight, with females becoming increasingly heavier with age.

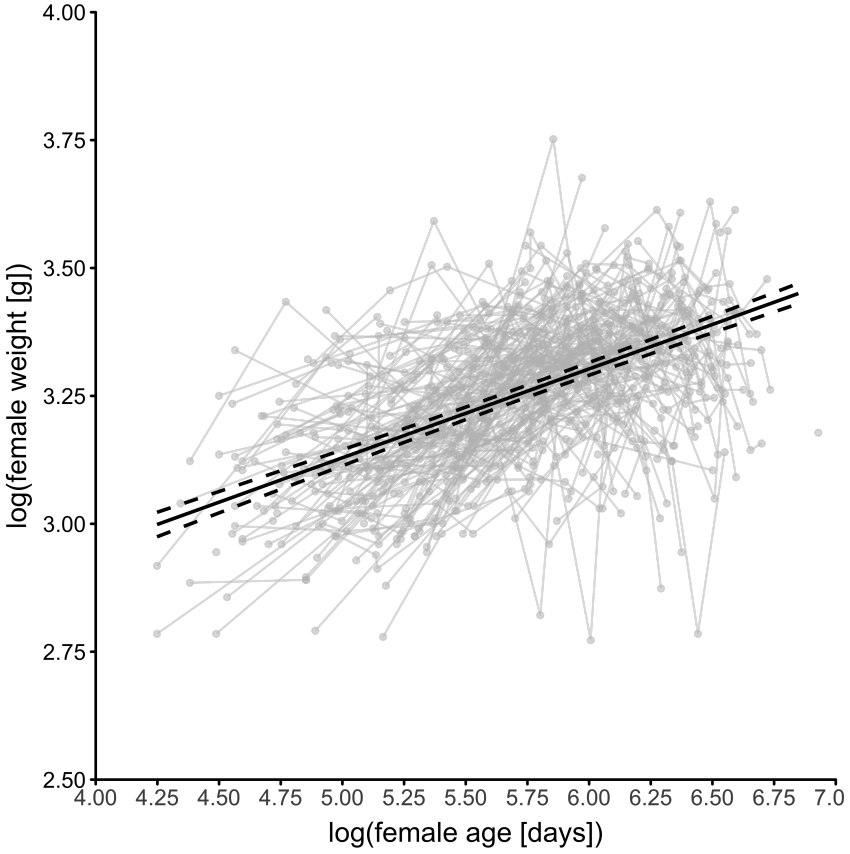


Figure 18: Female body weight in relation to female age (both plotted on a log scale). Plotted are mean estimates and 95%CI from a linear mixed model. In grey highlighted are raw data; weight measures of the same female are connected with lines (N=252 females).

*Sampling probability*

In a first step, we analysed a litter's probability to be found again in relation to its age, its size, the population size in the barn and the mean temperature during the month it was born. Only litters that were 10 days or younger when first found were included in the analyses (N=1039). We conducted the analysis at the level of the phenotypic litter (LitterID) and not the genetic litter (GeneticLitterID), because for pups or litters disappearing before sampling we did not know how many females contributed to a given litter (dead pups often are totally or partially eaten and leftovers are difficult to find in the barn, or they are already too decomposed to allow for successful genotyping). For the same reason, we were unable to correct for females potentially having several litters in the data set. With increasing age, litters had a higher probability to be found again, i.e. to survive until sampling (see Table 16 and Fig. 19, A). Similarly, larger litters were more likely to be found again (see Table 16 and Fig. 19, B). The population size in the barn or the mean temperature of the month in which a litter was born had no significant effect on a litter's sampling probability (see Table 16, the 95% CI overlapped 0).

Table 16: Model summary statistics for a litter's sampling probability

| | (Intercept) | population size | age first | ls first | mean temp | df | AICc | ΔAICc | w |
|---|---|---|---|---|---|---|---|---|---|
| **Model 1** | **0.451** | **–** | **1.053** | **1.433** | **–** | **4** | **1286.3** | **0** | **0.436** |
| **Model 2** | **0.447** | **–** | **1.048** | **1.428** | **0.139** | **5** | **1287.4** | **1.09** | **0.253** |
| **Model 3** | **0.442** | **0.221** | **1.048** | **1.435** | **–** | **5** | **1287.8** | **1.53** | **0.203** |
| **Model 4** | **0.440** | **0.180** | **1.044** | **1.430** | **0.127** | **6** | **1289.1** | **2.79** | **0.108** |
| Model 5 | 0.429 | – | – | 1.258 | – | 3 | 1335.8 | 49.53 | 0.000 |
| Model 6 | 0.423 | – | – | 1.252 | 0.152 | 4 | 1336.6 | 50.32 | 0.000 |
| ... | | | | | | | | | |
| Model 16 | 0.389 | -0.017 | – | – | 0.179 | 4 | 1409.6 | 123.36 | 0.000 |
| Averaged parameters | | | | | | | | | |
| Estimate* | 0.45 | 0.06 | 1.050 | 1.43 | 0.045 | | | | |
| Unconditional SE | 0.13 | 0.29 | 0.15 | 0.16 | 0.11 | | | | |
| Lower 95%CI | 0.19 | -0.30 | *0.75* | *1.12* | -0.16 | | | | |
| Upper 95%CI | 0.70 | 0.43 | *1.35* | *1.76* | 0.26 | | | | |
| Relative importance | | 0.31 | 1 | 1 | 0.36 | | | | |

Whether a litter was sampled or not (after first found at an earlier age) was used as response variable in a GLMM with a logit link function. Models within 4 AICc units of the best model are highlighted in bold and were used to calculate averaged effect sizes (*standardised on two standard deviations following Gelman (2008). Abbreviations: df, degrees of freedom; w, relative model weights. Significant confidence intervals (not crossing 0) are emphasised in italic. The year in which a litter was born was included as random term in all models. Factors included are defined as follows; population size: the number of adults in the population during the month in which a litter was born; age first: a litter's age when first found [days]; ls first: a litter's size when first found; mean temp: the mean temperature in the barn during the month in which a litter was born).

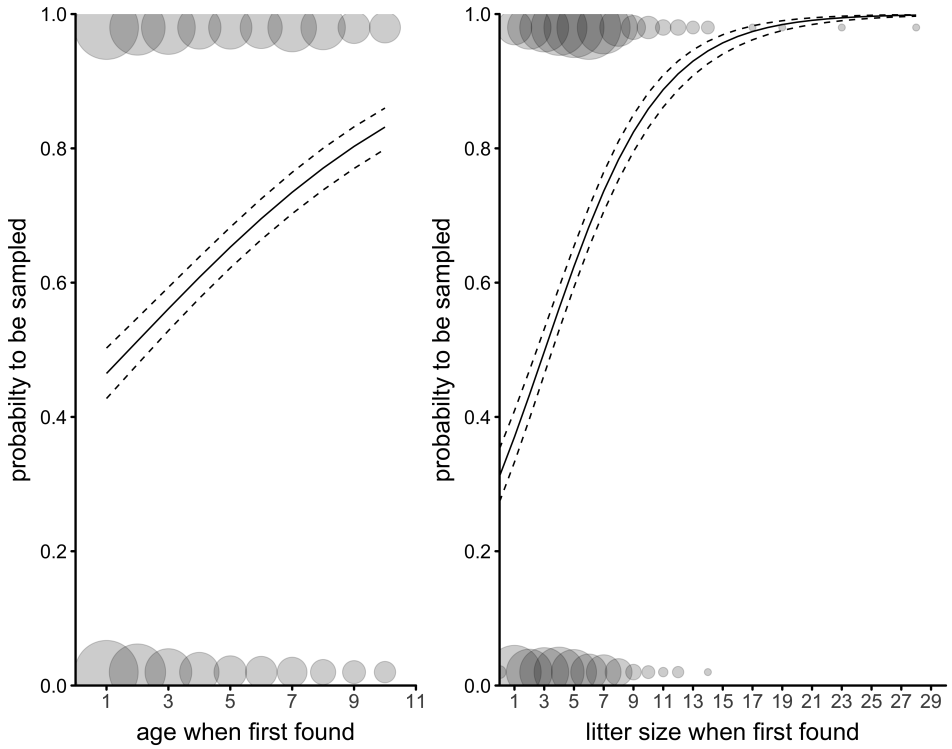


Figure 19: A litter's probability to be sampled in relation to **A)** its age and **B)** its size when first found. Only litters that were 10 days or younger when first found were included in the analyses (N=1039 litters). Plotted are model estimates (mean± SE) obtained from model averaging of binomial GLMMs. Darkgrey highlighted are raw data; the size of the circles illustrates the number of litters for a given value on the x-axis.

*Sample size for survival analyses*

Table 17: List of litters found between 2007 and 2011 used for survival analyses. Listed is the number of litters for the different categories. All of these litters were found at least twice (which allows to calculate survival).

| | | **first found** | | *total* |
|---|---|---|---|---|
| | | **solitarily** | **communally** | |
| **sampled** | **solitarily** | 161 | 33 | *194* |
| | **communally** | 68 | 78 | *146* |
| ***total*** | | *229* | *111* | **340** |

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